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PHYTOPATHOLOGY

VOLUME XIII NUMBER 3

MARCH, 1923

STUDIES ON PLANT CANCERS V.

LEAFY CROWN GALLS ON TOBACCO PLANTS RESULTING FROM BACTERIUM TUMEFACIENS INOCULATIONS[1]

MICHAEL LEVINE

WITH PLATES V TO VII

One of the most interesting results of the study of crown galls has been the discovery and careful study of those leafy forms (abortive shoots, etc.) which Smith has characterized as plant embryomata. The tobacco is probably one of the most favorable plants for the study of these leafy crown galls.

In studying the virulence of various dilutions of *B. tumefaciens* in tobacco, I have had under observation large numbers of leafy crown galls that were associated with and formed a part of the crown galls that developed on the stems, in the leaf axils, and on the midveins of the leaves, and have compared them with those of Bryophyllum (Levine 3).

Levin and Levine (1 and 2) and Levine (3, 4, and 5) claimed that the leafy shoot may result as a differentiation of the crown gall tissue. Further evidence for this view has been obtained. Such galls are to be distinguished in my opinion from the leafy crown galls which develop when an axillary or dormant bud is inoculated with *B. tumefaciens*. This type of leafy crown gall is distinctly different from those that develop on stem internodes or the midveins of leaves.

METHOD OF INOCULATION

In studying the virulence of various dilutions of *B. tumefaciens*, as reported elsewhere, I have described the various dilutions and the methods employed. A brief description of the methods used will suffice here. The plants were inoculated in the stem at the internodes, in the axillary buds, and in the midvein of the leaf. This was done by inserting a trocar .5 mm. in diameter to the depth of 2 or 3 mm. removing the tissue and replacing it by a drop of suspension of *B. tumefaciens* with the

[1] From the Cancer Division, Montefiore Hospital, Dr. Isaac Levier, Chief.

aid of a syringe. The more common method of inoculation, that is, pricking the plant with a sterile needle dipped into an emulsion of *B. tumefaciens*, was also used. But with the former method the quantity of fluid is measured more definitely than has heretofore been done by myself or other investigators. In the first or tocar method, a larger quantity of fluid can be injected. In general, the sizes of the crown galls obtained were not affected by the number of bacteria.

The inoculation of the uppermost axillary buds of decapitated plants was tried. Approximately two hundred plants were decapitated and immediately afterward were inoculated with *B. tumefaciens*. This was done by inserting a trocar into the axil of the leaf followed by injecting a drop of the culture. Often a slanting trocar incision was made extending from the center of the pith of the cut end of the stem diagonally to the axil of the leaf into which the *B. tumefaciens* suspension was introduced.

It is known that when a tobacco plant is decapitated, a number of axillary buds from the uppermost to the third or fifth dormant bud below are stimulated to develop, the uppermost assuming the position of the primary stem. It has been shown that injury or impairment of function, caused by the development of a crown gall near an axillary bud, will cause the development of the bud (Levine 5) and data, still unpublished for galls, on rubber trees shows that the death of the apical portion of the stem due to a crown gall is followed by the development of a number of axillary buds below the necrotized portion of the stem.

The stimulation of the buds brought about by decapitation is in a sense analogous to the stimulation of bud development brought about by the removal of a Bryophyllum leaf. The question was raised whether the removal of the leaf in Bryophyllum would act as an added stimulant to shoot formation when the notches of the leaves were inoculated. The conclusion reached was that *B. tumefaciens* generally inhibits the development of buds and leaf notches rather than stimulates them. In tobacco my results are the same as in Bryophyllum. Smith (12) finds that, "A distinct crown gall stimulus exists for the dormant buds of Bryophyllum."

In all, over one thousand inoculations were made on about 350 plants.

OBSERVATIONS

As noted, the results obtained by inoculating at three different points were compared, first, the midvein of the leaf, which in Bryophyllum gave only crown galls without differentiation of leafy shoots; second, the stem internode; and third, the axil of the leaf or the stem node.

INOCULATION INTO THE MIDVEIN OF THE LEAF AND THE STEM INTERNODE

For the study of leafy crown galls on the midveins of leaves, the tobacco is, in the opinion of the writer, the best material so far discovered.

The number of leafy crown galls so obtained is, however, relatively small. It approximates, however, under favorable conditions, the number of leafy shoots which appears on the stem internodes after inoculation. Of a great number of leaves inoculated in my cultures, only a small number reached maturity with their crown galls; the others failed to produce crown galls or withered before the crown gall was well developed. It must be remembered that by the time the young inoculated leaf becomes mature, its position is relatively further down on the stem, and more shaded so that in crowded cultures it may often wither and the development of the crown gall be thus interfered with. In all cases examined the growths on these midveins which later became leafy crown galls, began as crown galls of the ordinary type, the characteristic leafy shoot appearing later.

In inoculating the leaf midvein with *B. tumefaciens* by the trocar method a cylindrical opening is formed extending parallel with the axis of the midvein for a distance of 3.5 cm. With the development of crown gall, the tissue splits longitudinally. The growth of the crown gall is visible for two to three weeks before the diminutive leafy shoots are recognizable. The leaf structures first appear as small greenish white protuberances and then develop into recognizable small sessile leaves. It appears here as maintained by Levin and Levine, (2) and Levine (3, 4, and 5) that the leafy crown gall arises by a secondary process of differentiation. Just as the crown gall, in its earliest stages is made up of small cells, embryonic in appearance, and later becomes differentiated into various mature tissues, so by growth it may become differentiated into leaves and often modified stems and roots. Similar results are obtained when an inoculation is made by several pricks of the needle dipped into a culture of *B. tumefaciens*. Each incision is filled with a small crown gall of the globular type and later small leafy shoots develop from them. In all cases it was determined by careful observation that the leafy shoots appeared after the crown gall was well established. The formation of a cork-like layer and the appearance of a brown color over the surface of the crown gall indicates that a differentiate process is going on. Often, in the tobacco and especially in Bryophyllum, (Levine 3) it was noted that protuberances which were suspected of being capable of forming leafy shoots would spread out horizontally

and form flattened bodies not recognizable as leafy elements or roots. These would grow into large rounded masses and again give rise to protuberances which would in turn behave as described above. It appears that these protuberances can not be considered as definite embryonic leaves or roots, but are merely clusters of embryonic cells.

Smith's (10) figures (on plate 23) show similar types of development of the leafy crown gall. His figures show clearly a large globular crown gall from which the leafy structures develop. It appears that the size of these leafy shoots is determined in a great measure by the rapidity of the growth and differentiation of the crown gall. A good food supply will undoubtedly aid in the better development of these leafy structures. Most often the development of the globular crown gall is rapid and the differentiation of distorted woody elements cuts off the food supply so that the small leafy shoots dry up and die. It seems probable that the early death of these leafy structures on the crown galls is not a matter of senility, but is due to the limitation set on the available food supply by virtue of growth and differentiation of the crown gall proper.

Leafy crown galls on the internodes of the stem reach a much larger size than those on the midveins of the leaves. They are larger and the embryonic leaves which appear on them are more numerous and larger, than those on the midvein galls, but the same general sequence of events prevails. At times it appears that those on the stem have greater vitality. The leaves of these crown galls are relatively small and as compared with the normal and are always clustered. It seems that the greater the mass of tissue upon which the crown gall develops, the larger is its size and the greater is the differentiation of the crown gall tissues.

The leafy crown gall on the stem of the tobacco develops after the main mass of the crown gall tissue has been formed. In a number of instances, I have been able to obtain leafy crown galls, on the cut ends of stems of tobacco. These are similar to those shown by Smith (11) but in all cases a large mass of crown gall tissue is first formed on the cut end or within the body of the stem. This at first has somewhat the appearance of wound callus. A series of leafy crown galls obtained by inoculating with similar quantities of *B. tumefaciens* showing all stages in the development of the leafy shoot are shown in plate V, figures 1, 2, 3, 4, and 5.

Plate V, figure 1, represents a double crown gall at the internode of the stem of a tobacco plant inoculated in August 1921, and harvested in October 1921. The inoculation was made by perforating the stem by a trocar incision and *B. tumefaciens* was then introduced. Two con-

fluent crown galls developed. Each has a scaly surface and no indication of leafy shoots.

On the other hand the galls in plate V, figures 2, 3, 4, and 5 show a number of leaves varying in size and degree of development. Yet these plants were grown practically under the same conditions and inoculated similarly, and have produced equally large globular crown galls. In the first (Pl. V, fig. 2) the shoot on the crown gall consists of a leaf like protuberance "a". In plate V, figure 3, several leaf-like buds may be recognized while in 4 there has formed a structure which has a leaf and stem-like appearance. In plate V, figure 5, the galls produced by a perforating incision also have developed quite recognizable leafy bodies. It is of interest to note that while the globular crown galls are rather uniform in size, the size and number of the leaf-like elements vary.

A considerable number of plants showed swellings, comparable to the smooth galls described earlier (Levine 5 and 6). These galls are smooth in outline and covered by apparently normal epidermis. Sections through these crown galls show a hyperplasia of a particular tissue such as pith, wood or cortex, and the growth of the epidermis appears to keep pace with the growth of the internal tissues. The growth of the epidermis which covers the internal hyperplasia is not determinate, for it eventually cracks, necrotizes, and its scales form a part of the gall. An injury caused by a needle prick at the time of inoculation inhibits the growth of the epidermis at that point. The crown gall appears to grow through the opening and what appears to be callus tissue forms around the wound. Numerous cases of this phenomenon appeared in internodal inoculations of the tobacco.

The leafy crown galls so far described as noted form a group of crown galls which result from the further differentiation of the so-called globular type of crown gall.

INOCULATION OF STEMS AT THE AXILLARY BUDS, PLANTS NOT DECAPITATED

The effect of the inoculation of the axillary bud of the tobacco with *B. tumefaciens* was studied extensively. The growth that develops here is of two types. First, the globular crown galls formed are associated with a comparatively elongated branch-like growth which is undoubtedly the result of an abnormal development of the axillary bud. I have not made cultures to recover the organism from these shoots, but the presence or absence of the bacterium in the tissue would not necessarily indicate whether the parasite is the cause of its development. Never-

theless, this type of crown gall, when it occurs, is a distinct entity and may be distinguished from the leafy growths which appear on the surface of the globular crown gall by its position on the stem, namely, in the axil of the leaf. This axillary leafy crown gall as we shall refer to it may develop synchronously or appear somewhat later than the globular hyperplasia.

Plate VII, figure 10, and plate VI, figures 11, 12, 13, and 14 show a more or less graded series of axillary growth. In all cases, the axillary leafy shoot is associated with the crown gall, often growing through it; more commonly the leafy growths appear on a dwarfed atypical stem as shown in plate VII, figure 10, and plate VI, figures 11, 12, and 13. It not infrequently occurs that the axillary shoot appears to be quite normal. This occurs when the inoculation is made at the side of the axillary bud (Pl. VI, fig. 14). The development of this shoot is not to be construed as directly caused by the organism, but rather indicates a vascular disturbance affecting the distribution of food, thus calling forth this growth, possibly in the same way that decapitation would do.

It is of interest to note that in plate VII, figure 10, the crown gall appears to be an abortive stem from which aberrant leaves appear, having a more or less cyclic arrangement. In plate VI, figure 12 the branch marked "a" can be recognized as the axillary shoot. It is stunted, yet the arrangement of the leaves can be definitely recognized. Plate VI, figure 11 represents a similar condition; "b" is the axillary shoot the base of which is surrounded by globular crown gall which differentiated into leafy appendages. Plate VI, figure 13 shows still further the size to which the axillary leafy crown gall may develop.

The most striking characteristic of the growths of this type which arise from the axillary bud when inoculated with *B. tumefaciens*, is that the structures so formed are never as well developed as an axillary shoot arising from a decapitated stem, but are dwarfed, fasciated and abnormal in appearance. The leaves are often pale green and in a measure resemble the leaves of the other type of leafy crown gall. These leafy structures vary in size and development and form a series of growths varying from an typical branch to one almost normal in appearance (Pl. VI, fig. 14). These leafy crown galls may be regarded as the typical axillary leafy crown gall.

That *B. tumefaciens* does not always cause the development of this axillary type of leafy crown gall may be seen from the great number of inoculated axillary buds which do not produce these leafy shoots. This constitutes the second type of cases. It appears in the case that the

inoculation of *B. tumefaciens* into the axillary bud may inhibit partially or completely the development of the dormant bud. In plate V, figures 6, 7, 8, and 9, we have a few of the large number of cases of this sort obtained by inoculating the axillary buds. Large globular crown galls have been produced, while the dormant buds have failed to develop. Such types of development predominated my cultures of Bryophyllum (Levine 3). It is perhaps possible that under these conditions the entire bud rudiment is deformed and is either given over to crown gall cell formation or is engulfed by the rapidly growing cells that form the crown gall. In all these cases, the leafy crown gall of the differentiated type, that is, the form with small leaves coming from the surface of the crown gall may also be present.

The development of the axillary leafy crown gall may be due at first to a stimulation brought about by increasing the food supply. The young crown gall cells may be responsible for this by stimulating the transportation of food supply, thus causing the development of the axillary bud. The development is then retarded and the shoot is dwarfed or stunted when compared with the shoots from axillary buds stimulated by decapitation. A careful study of a large number of these growths confirms the opinion that the crown gall organism while it may indirectly stimulate the development of the dormant bud, prevents its full normal development.

INOCULATION OF THE STEM AT THE AXILLARY BUD OF DECAPITATED PLANTS

My further test of the effect that the crown gall organism has on an axillary bud when stimulus such as decapitation is added, gives results similar to those I have already described for Bryophyllum leaf notches. In the latter case, the leaf notches were stimulated to shoot production by removing the leaves from the plant and placing them in moist soil. The control leaves uninoculated produced at the leaf notches large normal shoots, while those leaves inoculated at their notches with *B. tumefaciens* produced relatively large crown galls and diminutive or no shoots at all. Smith's (12) figures also show this to be the case for leaves not detached from the plant. Whether there is a summation of the stimuli to growth resulting from the two sources, viz. decapitation and inoculation, is not altogether clear from either my results or those of Smith. It is not easy to make exact comparison of the number and exact character of the growths initiated under the two sets of conditions. It is of course perfectly clear that the final influence of the organism is to dwarf and deform the growths which do start in the inoculated regions

but it is quite possible that the stimuli to cell growth and division from the two sources are summated though the further effect of the organism when present is to prevent the harmonious biogenetic interaction of the cells produced which would lead to the formation of a normal leafy shoot. That the inoculated buds produce a smaller total growth in the end may be merely due to this resulting malformation which prevents normal assimilation and transportation of food stuffs.

In the case of the tobacco, decapitation leads to the development of a number of axillary buds so that at the end of the summer season a large decapitated plant may produce from two to five large branches arising from the upper axillary buds. The inoculated axillary regions produce crown galls with malformed branch-like growths. The elements of the axillary shoots are recognizable but the characteristic normal development does not appear. The shoots are generally stunted; the internodes are short, and the leaves appear to be aberrant, small and of a pale green color. The mechanical injury concomitant with the inoculation may also have a part in disturbing the bud rudiment. A number of small branches may appear with the crown gall. These leafy shoots appear to develop synchronously with the crown gall and form a component part of it.

Plate VII, figure 15 represents a case in which the axillary shoot developed but slightly after inoculation. Here the crown gall "b" seems to partially surround the cut end of the primary shoot "a," which has been cut close to the axil of the leaf; "c" represents the development of the axillary bud. Compare these aberrant branches with the diameter of the branch arising from the axillary bud "d" uninoculated. For purposes of photography the shoot "d" was removed. The diameters of the two are sufficient to show that the uninoculated axillary bud develops into a normal branch, which when highest on the stem, as is well known, assumes the position of the primary shoot, while the inoculated axillary bud produced here a large crown gall with a small stunted shoot ("c").

In another plant shown in Plate VII, figure 16, the largest in my cultures, similar results were observed. The axillary leafy crown gall produced several shoots all of which were smaller than the five axillary leafy crown gall type, dwarfed and pale green. The leaves were attenuated, also pale green in color and dried at the tips.

The crown gall in this case was very extensive, the plant bearing it was the largest that appeared in these cultures. This is in line with the evidence (Levine 6) that the better the health of the host, the larger the size of the crown gall. The gall is covered by abortive leafy outgrowths.

This combination of the leafy crown galls and the axillary leafy crown gall, an aberrant development of the axillary bud often appeared.

It is as noted very obvious that while inoculation with *B. tumefaciens*, like decapitation, acts as a stimulant to cell growth and division, the parasite when present regularly leads to rather degenerate further growth and poor development as compared to the growth of an axillary bud due to stimulation by decapitation without inoculation.

SUMMARY

I. (1) Two types of leafy crown galls are distinguished in the tobacco plant when inoculated with *B. tumefaciens*.

(2) The first type has already been described and figured as globular in form and covered with small leaf-like structures which result from the differentitation of the crown gall tissue. Such galls appear on the inoculated midveins of the leaves and on the stem internodes of tobacco.

(3) The second type of leafy crown gall has been referred to in this paper as the axillary leafy crown gall. It appears often when the axil of the leaf is inoculated with *B. tumefaciens*. The axillary leafy crown gall consists of rather elongated stem as compared to the leafy crown galls of the first type, and results from the aberrant development of the axillary bud.

(4) It is not certain that the growth of this leafy shoot is due directly to the presence of *B. tumefaciens*. The stimulus to growth may be the result of a mechanical modification of the food supply incident to the development of the crown gall tissue.

(5) The axillary leafy crown gall is small and atypical as compared to the shoot from an axillary bud stimulated to growth by decapitation.

(6) An added stimulus such as decapitation does not increase the size of the axillary leafy crown gall when the axillary bud is inoculated with *B. tumefaciens*.

DESCRIPTION OF PLATES

Plate V. Figures 1, 2, 3, 4, and 5 show a series of crown galls produced by the inoculation of the internodes of the stems of tobacco with *Bacterium tumefaciens*. The crown galls show a graded series of leafy developments on the globular mass of the neoplastic tissue. In figure 1 the leafy shoots are barely visible. In figures 2, 3, and 4 the size of the leafy protuberances are larger. In figure 5 the apical portion of the crown gall on opposite sides of the stem are profusely covered with well differentiated leafy shoots.

Plate V. Figures 6, 7, 8, and 9, show a series of crown galls produced by inoculating the axil of the leaves with *B. tumefaciens*, the development of the axillary bud in each case is inhibited.

Plate VI. Figures 11, 12, 13, 14 and figure 10 plate VII show crown galls in the axils of leaves with various stages in the development of the axillary bud. The familiar globular portion of the crown gall is covered with leafy shoots.

Plate VII. Figures 15 and 16 show the extremes of the effect of inoculation of the upper axillary bud of a decapitated tobacco plant with *B. tumefaciens*. In figure 15 the axillary bud development represents a large globular crown gall through which a small aberrant leaf-like stem developed; the leafy crown galls of the differentiated variety may also be seen at the base of the gall. Figure 16 represents a longitudinal section of a tobacco plant through the crown gall. The cut end of the stem is covered with differentiated crown gall tissue consisting of numerous leafy shoots. The axillary bud appears to have grown through the mass of crown gall tissue into two aberrant stem like structures.

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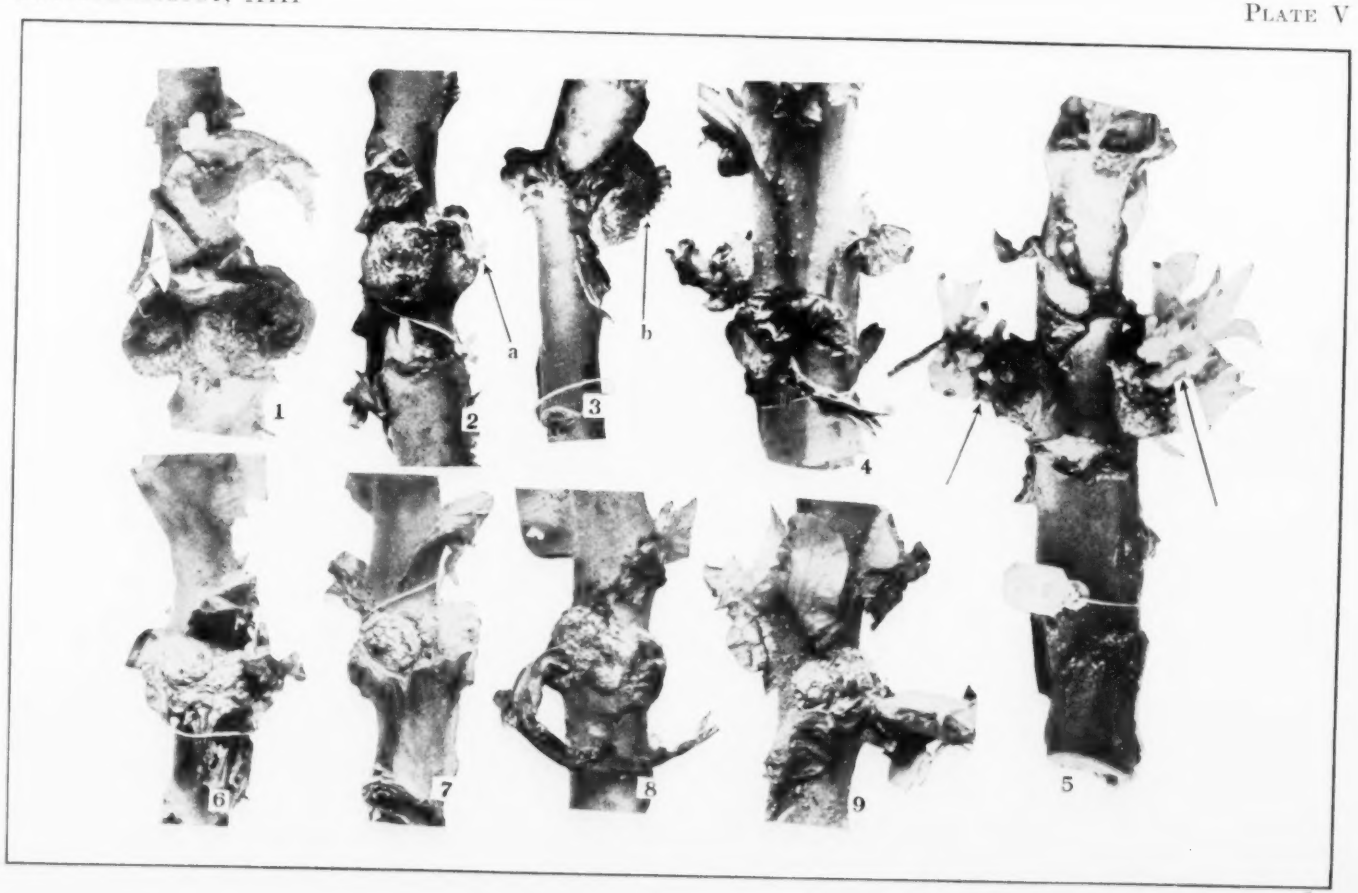
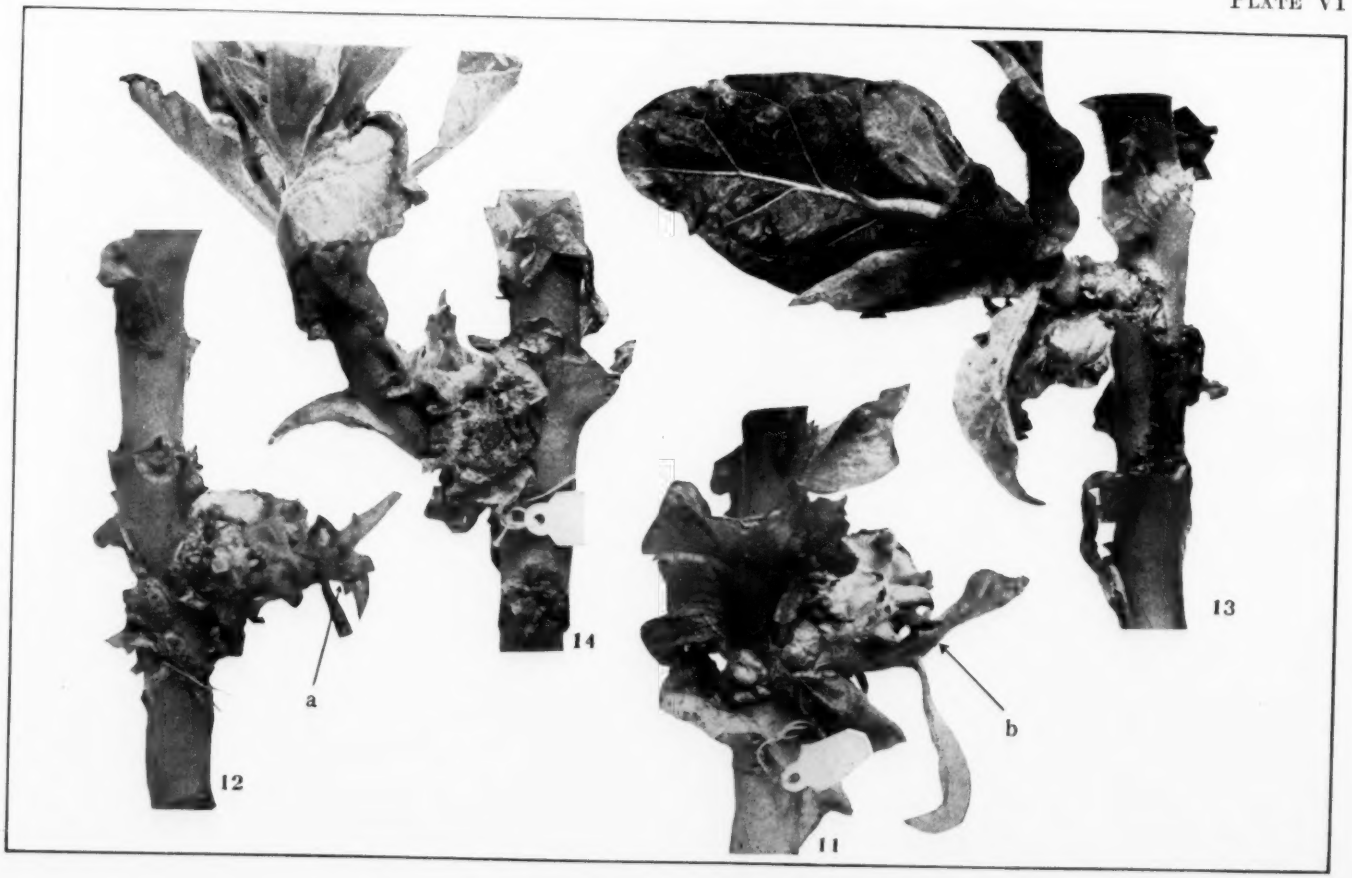
PLATE V

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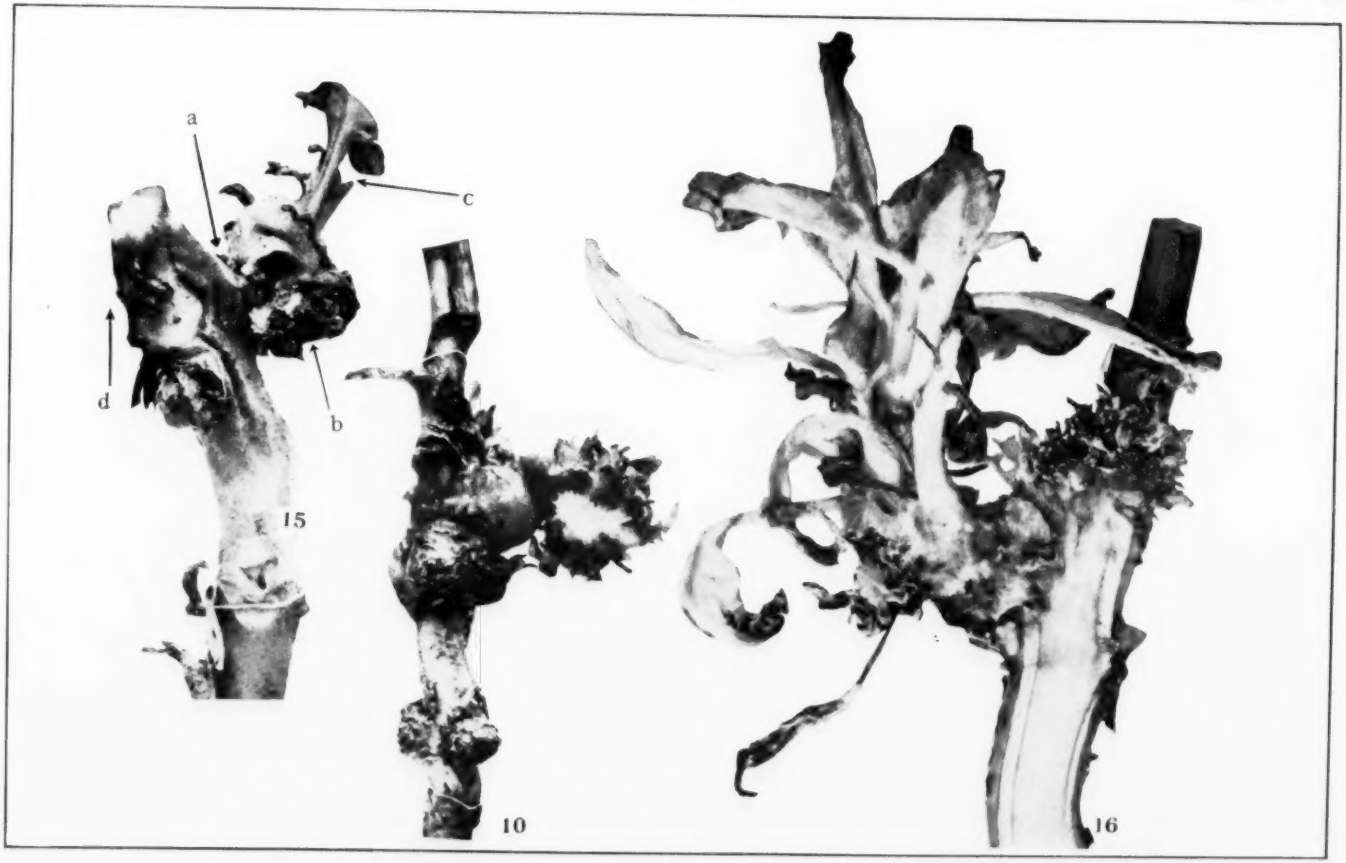
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16

THE SPHAERULINA LEAF SPOT OF CLOVER

E. F. Hopkins[1]

With Plates VIII and IX and Three Figures in the Text

This leaf spot was first observed by the writer in the spring of 1920 on the campus of the University of Missouri. The resemblance between it and a leaf spot of bur clover caused by a species of Pleosphaerulina previously studied by the writer in Alabama is very striking and suggested the possibility that this also might be caused by a species of this same genus. A search of the dead clover leaves near infected plants showed that many of them bore perithecia similar to those of Pleosphaerulina. The ascospores, however, showed no longitudinal walls as in the latter. The species is, therefore, a Sphaerulina and has been determined by the writer as *Sphaerulina trifolii*. E. Rostr. From a close study of the literature it appears that the disease has not been previously reported in America and for this reason it will perhaps be of some value to give an account of observations and experiments made with this leaf-spot since it was first found.

Evidence that the disease was present in this locality at least as early as 1902 was obtained on examining a specimen of *Trifolium repens* in the phanerogamic herbarium of the University of Missouri. It was noted that some of the lower leaves of this specimen bear the typical minute young lesions of the Sphaerulina leaf-spot. A microscopical examination of these small spots showed them to be the same as those described below and also to have usually near their center the empty walls of the ascospores from which the lesions had developed. Thus far the disease has been found on white clover (*Trifolium repens* L.), alsike clover (*T. hybridum* L.), and on red clover (*T. pratense* L.). Data from inoculation experiments indicate that mammoth clover (*T. pratense perenne*) is also susceptible, white sweet clover (*Melilotus alba* Lam.) and yellow sweet clover (*Melilotus officinale* Willd.), bur clover (*Medicago maculata* Willd.), and alfalfa (*Medicago sativa* L.) are probably immune. The disease has been found most commonly by the writer on white clover in the vicinity of Columbia, Missouri. Other collections have been made at Rocheport, Clayton, St. Charles, and Blue Springs, Missouri; Ithaca, N. Y., and Godfrey, Ill. The writer has found it in nearly every growth of white clover examined. The loss caused by it is probably not great, although a considerable amount of defoliation may take place under favorable conditions.

[1] Published with the approval of the Director of the Missouri Experiment Station.

SYMPTOMS

The disease is exhibited at first as minute black lesions on the leaf blades, petioles, and stipules. These lesions are very small and easily distinguished from spots caused by *Phyllachora trifolii* (Pers.) Fckl. At this stage the leaf has the general appearance of being peppered. The spots are slightly depressed especially on the petioles and on the under sides of the leaves. Both the upper and lower sides of the leaf usually bear lesions. Leaves have been found frequently, however, with lesions only part way up on the petiole and others with only the petiole and under side of the leaflets infected. The presence of numerous lesions of this sort often causes the leaflets to turn yellow and fall. When the leaflets turn yellow the dark lesions stand out in strong contrast. Even if there are but a few young lesions on a leaf they are more readily distinguished by holding it between the eye and a light. Such young lesions are shown in plate VIII, figure 1, photographed by transmitted light. Later these small spots enlarge and have a light brown to gray center surrounded by a dark reddish brown margin as shown in plate 1, figure 2. The perithecia may sometimes be seen with the unaided eye in these older lesions. By holding the leaf up to the light and examining it with a hand lens the perithecia stand out clearly as small bright bodies in which one may often distinguish the ostiola. Under conditions of continued high humidity the whole of the infected leaflet may become water-soaked in appearance and develop large numbers of perithecia throughout the tissue. In the case of red clover, perithecia develop readily on the stipule lesions.

Besides the parts mentioned above, lesions occur also on the peduncle, calyx, and corolla. Infection on the calyx seems significant in that it points to a means by which the seed may become infected and thus transmit the pathogene. Young lesions on the petioles and peduncles are usually elongated vertically but are otherwise similar to those on the leaf blades. Infections have not been found by the writer on the stems of either white or red clover although numerous lesions have been observed on peduncles and petioles in close proximity thereto, so that although it seems that the stems must have been exposed to ascospore discharge they did not become infected. Abundant infection has been found on the stems of alsike clover. In one instance minute lesions on red clover stems which did not appear to be typical of the Sphaerulina disease were observed. Isolations from these lesions, moreover, gave rise in all cases to pure cultures of another organism.

ETIOLOGY

Nomenclature. The fungus was determined by the writer to be a species of Sphaerulina and was then compared with herbarium specimens of *S. trifolii* E. Rostr. on *Trifolium repens* (No. 790 Sydow Mycotheca germanica). The two are identical both as to the lesions produced on the clover leaves and as to the morphology of the organism. The limits of ascospore length as given by Rostrup[1] are somewhat smaller than those determined by the writer for his own material. The former places the limits as 32 to 33 by 12 to 15 μ while measurements of ten ascospores of material collected here vary from 30.4 μ to 39.5 μ with an average of 35.1 μ. At the same time the spore width was found to vary from 12.2 μ to 15.1 μ with an average of 13.2 μ. As it is seen this agrees very well with Rostrup's limits. As shown in figure 1, B, the mature asci are considerably longer than wide. Rostrup gave their measurements as 50 μ in diameter and did not give values for the length and width. The measurements of asci gave a variation in length from 64 μ to 71 μ with an average of 72 μ and in width from 33 μ to 50 μ with an average of 43 μ. Measurements of the herbarium specimen mentioned above were made and are listed below along with Rostrup's measurements and measurements made from the writer's own specimens.

	Present collections	No. 790 Mycotheca Germanica	Rostrup's measurements
Perithecia	80–125 μ	98–102 μ	Not given
Asci	64–71x33–50 μ	47–59x30–41 μ	50 μ diameter
Ascospores	30.4–39.5x12.2–15.1 μ	25.2–29.4x11.2–12.6 μ	32–33x12.15 μ

It seems evident therefore that the ascomycete under consideration is the same that Rostrup observed on white clover in Denmark and is *Sphaerulina trifolii.*

Morphology. The mycelium of this species as it occurs in infected tissue is usually rather large in diameter, is densely granular and frequently shows swellings. On potato agar plates it is somewhat the same but perhaps more uniform. The perithecia are outlined in figure 1, B. They are flattened slightly on the vertical axis and have relatively large ostiola. The perithecial walls are somewhat smoky in appearance but are very thin so that when the contents are mature the asci and ascospores may be seen within them. As mentioned above perithecia

[1] Rostrup, E. Mykologiske Meddeleser (VIII). Spredte Iagttagelser fra 1897–1898. Bot. Tidsskr. **22:** 254–276. Illus. 1899. Bibliographical footnotes.

appear as small bright bodies when the older lesions are viewed by transmitted light with a hand lens. They are not beaked. The ascus when young is pear-shaped as shown in figure 1, B, and at the base has a much thickened wall composed of a homogeneous hyaline substance. This wall gradually becomes thinner as it approaches the larger end of the ascus in which the young ascospores are borne. Later when the spores are mature this thickened part of the wall is reduced and appears as in figure 1, C.

The ascospores are almost invariably four celled with three cross walls although a few exceptions (e. g. two and three celled spores) have been found by the writer. Some of the spores are symmetrical but a large number show a bulging out of one of the central cells. The mature spores are filled with granular protoplasm which often contains a number of large oil drops (Figs. 1, C and 2).

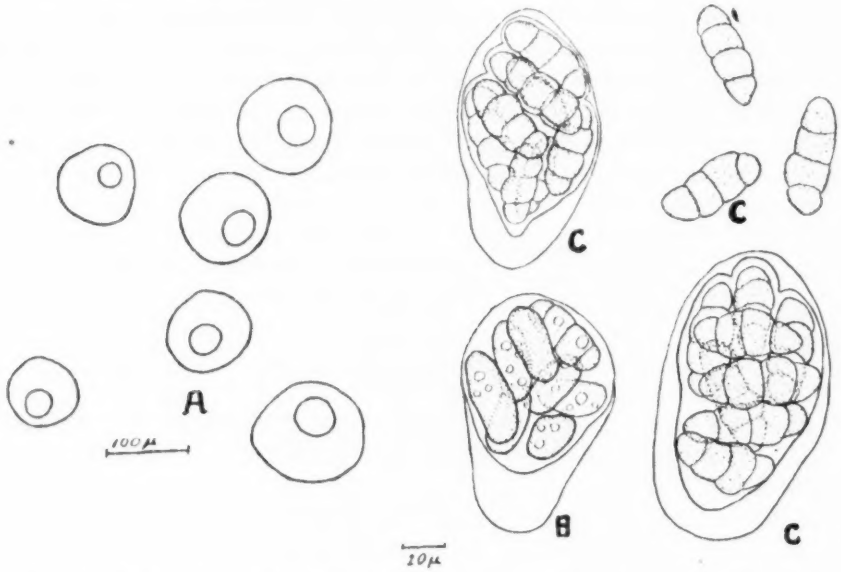

FIG. 1. Sphaerulina trifolii: A. Perithecia from a white clover leaf. B. An immature ascus from white clover. C. Mature asci and ascospores from white clover Camera lucida drawings.

Isolations. A large number of isolations were made from leaf blades, petioles, stems, calyces, and peduncles which showed the small lesions, and in every case the same characteristic growth was obtained on potato agar as shown in plate IX figures 1 and 2. This growth was also identical with colonies from the tissue of larger lesions and from single ascospores.

In two instances cultures of *Colletotrichum trifolii* Bain were obtained with Sphaerulina. Thus far no mature perithecia have been obtained in pure culture. The fungus makes a good, but slow growth on potato agar. At first the colony consists of white mycelium only, but after a few days spherical white bodies which soon turn shiny black appear at the center and finally large numbers of them are formed over the surface of the colony up to within a few millimeters of the advancing margin. At this time the fungus forms a tough membrane on the surface of the agar

FIG. 2. Sphaerulina trifolii: Ascospores from the surface of an infected leaf. Camera lucida drawing.

and there is but little aerial mycelium present. The general appearance of the colony is black with a white border. A large number of microscopic examinations of these black structures failed to show any with asci, ascospores, or pycnospores. The wall is membranaceous and is made up of cells similar to the walls of the perithecia found in nature but with no ostiolum. The contents are granular and frequently contain what appear to be oil globules. In cultures on sterilized clover leaves in large tubes and preparation dishes the same black bodies were formed abundantly in the leaf tissue. In this instance as before, none of them contained mature asci. However, in some, a number of ovate hyaline structures which may have been inmature asci were observed.

Cultures on potato agar when actively growing have a pleasant fruit-like odor not present in cultures of *Pleosphaerulina briosiana* to which they are otherwise quite similar. In several isolation experiments in which the growth was at first not typical the presence of the organism was indicated by this odor. Later the cultures developed normally. A strong acid reaction does not prevent growth and this fact is of value in isolating when bacteria are present. In the following experiment actively growing mycelium from a pure culture of the organism was planted on plates containing 1, 2, and 3 drops of lactic acid respectively, per 20 cc. of potato dextrose agar.

TABLE 1

Growth of Sphaerulinia trifolii on acidified potato dextrose agar.

Drops of 50 per cent lactic acid	pH (approximate)	Growth in diam. mm.				
		30 hrs.	2 days	3 days	4 days	6 days
1	4.5	13.0	15.5	17	19.0	22
2	4.0	11.0	13.5	15	16.5	19
3	3.8	7.5	11.0	11	14.0	10

Even with three drops of the acid there is fairly good growth after 30 hours although the growth is only about one half that in the least acid culture. It is evident from the table that after a time the relative difference between the growth of the cultures gradually disappears. This is probably due to a change in reaction in the more acid culture plates which makes them more favorable for growth.

Pathogenicity. Difficulties were experienced in obtaining infection with mycelium from pure cultures of *Sphaerulina trifolii* because of the drying out of the inoculum before the pathogene had become established. However, in one experiment with a single ascospore culture (No. 32) and a culture isolated from infected tissue (No. 30) strong infection was obtained on mammoth clover, medium infection on white clover, and slight infection on red clover. Reisolations from the infected leaves were successful.

As the lesions produced in the above experiment were not typical an attempt was made to obtain more natural infection. Two pots of red clover plants free from leaf spots were used. Over one of the pots was placed a bell glass in the top of which were attached white clover leaves which bore large numbers of perithecia. As a control the other pot of

plants was covered with a bell glass in which no perithecia were placed. Both bell glasses were removed at the end of about 76 hours. No difference was noted at the time of uncovering, but two days later the plants exposed to the perithecia showed abundant minute lesions identical with those found in the field. After two days more, a careful inspection was made of each leaf. Of 70 leaves in the pot exposed to ascospore discharge, 52 or 74.3 per cent were infected, while none of the 60 leaves in the control plants were infected. Successful isolations of Sphaerulina from the spots produced in this manner were obtained. A microscopical examination of the lesions showed at their centers the presence of ascospores usually devoid of contents as is the case in field infection.

The experiment was repeated but this time 8 different species of legumes were used. They represented three genera: Trifolium, Medicago, and Melilotus. Plants in one pot of each were inoculated and those in one pot used as a control. The bell glasses were placed over the plants on a cold cloudy afternoon. No lesions were noticed on any of the plants by the next day, but the morning following (41 hours after exposing to the perithecia) definite and characteristic lesions were found on the leaves of all inoculated plants while none of the control plants showed any lesions. In table 2 the species are arranged in order of the relative amount of infection, beginning with mammoth clover which was most severely affected and ending with bur clover which was least infected. The numbers in the third column indicate approximately the relative amount of infection on the leaves on the basis of 10, which represents the most severely infected.

The bell glasses were removed at the end of 48 hours and the plants were placed under a cheese cloth shade in the greenhouse to protect them from the heat of the sun. The lesions on the species of Trifolium continued to develop in size while those on the alfalfa, bur, and sweet clovers even after 8 days did not appear larger than they were when first observed.

From the above experiment and also from the first inoculation experiment it may be concluded that mammoth clover is the most susceptible, while red and white are about equally so under conditions of our experiments, and alsike less susceptible. The writer has not had the opportunity to study mammoth clover in the field. However, under field conditions white clover is much more severely attacked than red clover. The species of Medicago and Melilotus are probably not naturally host plants for this organism, as the spots did not develop further than the pin point stage as they did in the case of mammoth, red, white and alsike

clovers. While a pure culture was not used in the last two experiments the writer considers that the pathogenicity of the organism is demonstrated.

Seasonal history. In the spring of 1922 as soon as the white clover began to grow it was inspected almost daily for the Sphaerulina leaf spot. No infection was found up to March 13. Previously there had been little precipitation but from March 13 to 14 there fell about four inches of rain and on the 17 one center of infection was found, and the next day others. As a check in the diagnosis frequent isolations and

TABLE 2

Results obtained on inoculating various species of Trifolium, Medicago, and Melilotus with Sphaerulina trifolii.

Host	Relative infection	Remarks
Mammoth clover (*Trifolium pratense* L.)	10	Lesions very abundant and prominent
White clover (*Trifolium repens* L.)	7	Lesions abundant and strong.
Red clover (*Trifolium pratense* L.)	7	A large number of lesions much more prominent than in the case of bur clover.
Alsike clover (*Trifolium hybridum.*)	5	A few scattered lesions.
Alfalfa (*Medicago sativa* L.)	3	Spots few but prominent.
White sweet clover (*Melilotus alba* Desr.)	3	Spots few but prominent.
Yellow sweet clover (*Melilotus officinalis* L.) Lam.	3	Few scattering lesions but more prominent than those on bur clover.
Bur clover (*Medicago maculata* Willd.)	2	A few minute lesions.

microscopic examinations were made. At this time a search was made for the source of these primary infections. A number of old dead leaves were found at the base of some of the infected clover plants. These leaves were covered with perithecia containing asci and mature ascospores. As they seemed to be in active condition there is no doubt that they were responsible for the first infections of the year. It seems plausible that they remained dormant until the heavy spring rains caused the asci to shoot their spores and initiate infections on healthy leaves. As mentioned under symptoms these minute spots gradually enlarged and turned light brown at their center where perithecia are produced. These perithecia then furnish more inoculum which causes infections throughout the season. Up to the present time no conidial

stage has been found to be connected with the species. Although several of the Fungi Imperfecti found on clover leaves have been cultured, the cultures do not at all resemble those of *Sphaerulina trifolii*. The writer has not attempted to determine to what height the ascospores are shot from the perithecia, but the following experiments show that they are shot upward at least one half centimeter. A leaflet containing perithecia was moistened with sterile distilled water and attached to the under side of the cover of a petri dish which had been previously poured with

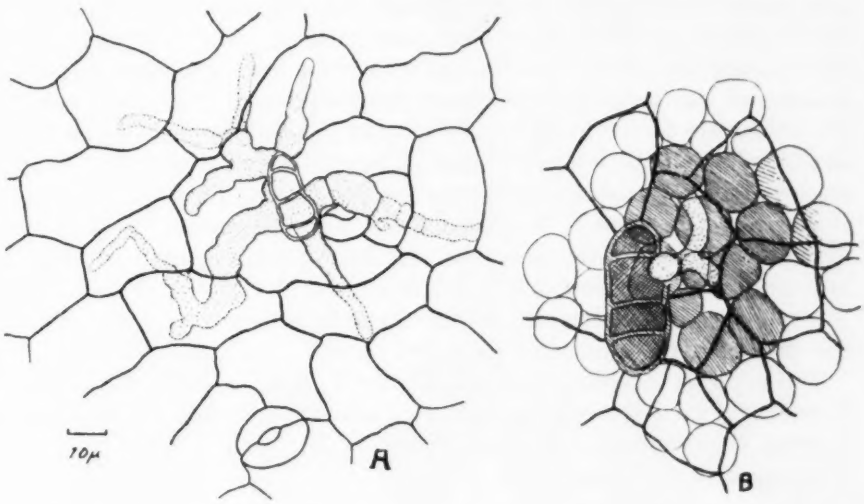

FIG. 3. Sphaerulina trifolii. A. Penetration of epidermis of white clover leaf. Camera lucida drawing. B. Details of early stage of infection on leaf of white clover. The ascospore and palisade cells in the immediate vicinity have turned brown.

potato agar. The plate was inverted and left over night and by the next morning (about nineteen hours) germinating ascospores were found on the surface of the agar plate directly above the leaf. The inoculation experiment discussed above shows that the perithicia readily expel their spores when plenty of moisture is present.

The fact that lesions have been found on the calyx and corolla indicates that seed infection may take place and this would account for the general distribution of the pathogene. Of a large number of commercial samples of white clover seed examined, mycelium was found in practically every instance in some of the seed coats. No successful isolation cultures were obtained from these seed to establish the identity of the

fungus although very short periods of disinfection were used. It was finally concluded that the samples were old and the mycelium was dead. Seed will be collected from well infected clover plants and an attempt made to settle this question.

Pathological relations. Soon after the ascospores come into contact with the leaf surface they germinate and send germ tubes directly through the epidermis. The germ tubes may grow for some distance from the spore on the surface before penetrating the epidermis, as in figure 3, A, or they may penetrate directly as in figure 3, B. No definite appressoria have been made out although the germ tubes are frequently swollen above the point where they enter the leaf. The empty walls of the ascospore persist for some time after infection has taken place perhaps because of its thickness. In about thirty-six hours after inoculation the lesions are visible to the eye because of the dark brown color which developed about the infected region. The spore itself takes on a brown color and also the palisade cells near the penetrating germ tubes. This is illustrated in figure 3, B. Finally, the color becomes so deep as to obscure the details of the infected spot. As the spot enlarges the center becomes thin and brown due to the death and drying out of the cells. The margin of the lesion still retains the deep brown color which gives the characteristic appearance as shown in plate VIII, figure 2.

The writer is indebted to Miss Marjorie F. Warner, Librarian of the Bureau of Plant Industry, for the exact citation of the article containing Rostrup's description of *Sphaerulina trifolii*.

DEPARTMENT OF BOTANY,
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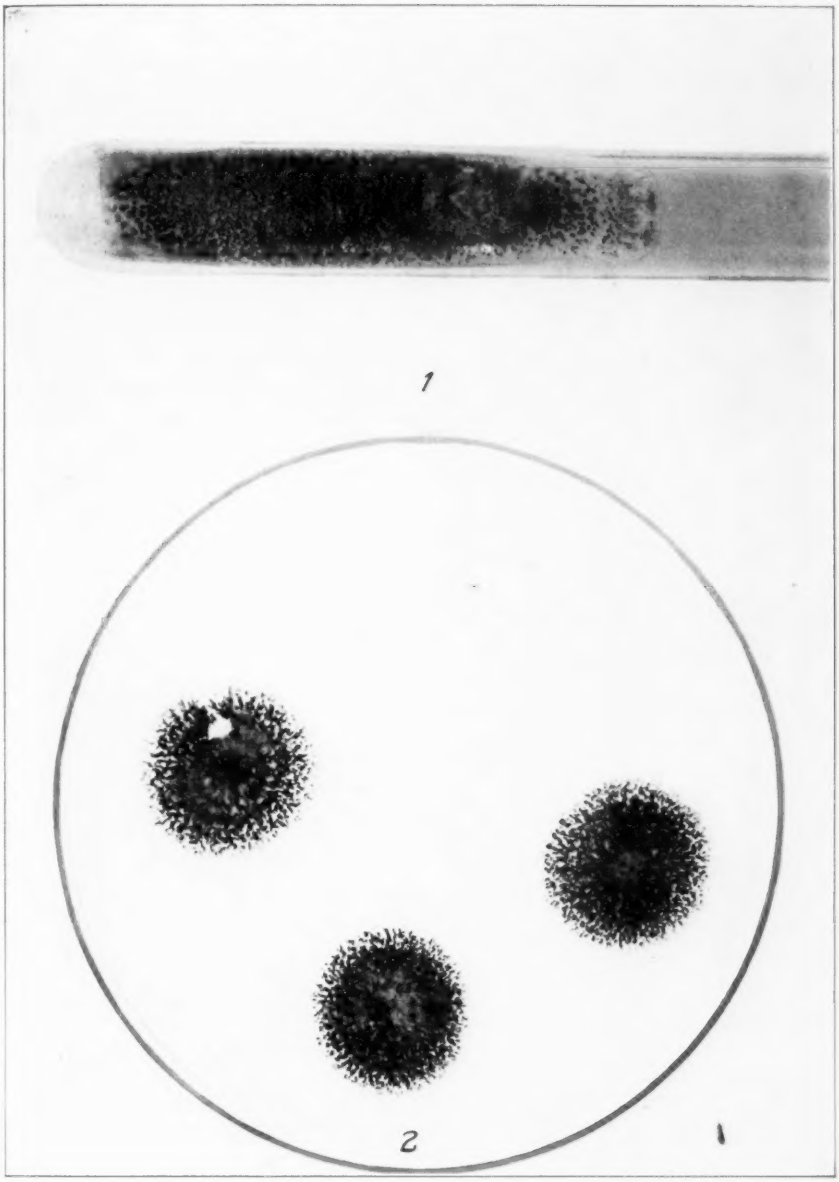
SPHAERULINA LEAF SPOT OF CLOVER.

FIG. 1 Sphaerulina leaf-spot on leaflets and leaf petioles of *Trifolium repens*. Early stage.

FIG. 2. Sphaerulina leaf-spot on *Trifolium repens*. Adavnced stage. Note the color about the margins of the lesions.



SPHAERULINA LEAF SPOT OF CLOVER

FIG. 1. Potato agar slant culture of *Sphaerulina trifolii*.

FIG. 2. An isolation plate culture of *Sphaerulina trifolii*.

STUDIES ON UROCYSTIS TRITICI KOERN., THE ORGANISM CAUSING FLAG SMUT OF WHEAT

R. J. NOBLE[1]

WITH PLATE X AND TWO FIGURES IN THE TEXT

Studies on the physiological relationships of *Urocystis tritici* Koern. have always been considerably hampered by the fact that the conditions necessary for spore germination were not known.

This apparently also is true for a number of other smuts. On account of scanty germination of the fungous spores, the study of the organism has frequently been limited to inoculation experiments with infested soil. The present investigations were directed toward a determination of the conditions which would result in more vigorous germination of the flag smut spores.

GERMINATION STUDIES

For several years McAlpine (3) made observations on the germination of spores of *U. tritici*. He used tap water in his tests, and records germination which varied from a "small proportion" in material one month old, to 40 per cent in material which apparently was four or five months old.

Early studies by the writer indicated that the spore germination was very capricious. Material from Australia and the United States was used in a number of preliminary tests, but, under a series of varied conditions in trials extending over periods which ranged from several days to several weeks, only a few spores germinated.

MATERIAL AND METHODS

The spores used in the following series of tests were obtained from the wheat varieties "Bobs" and "Federation," grown in the United States Department of Agriculture greenhouse, Arlington, Virginia, and collected in March 1921. When the results recorded below were obtained, this material was at least six months old; and the spores still germinated normally when they were fourteen months old.

[1] Ben Fuller Research Scholar of University of Sydney, Australia, and Graduate Student of the University of Minnesota.

The writer wishes to acknowledge his indebtedness to Professor E. C. Stakman of the Department of Plant Pathology, University of Minnesota, for advice and helpful criticism, and also to the United States Department of Agriculture for material and for granting the necessary facilities for the investigations.

It was found that most uniform results could be obtained by the use of Syracuse dishes. The spores were sown on the surface of from 2 to 3 cc. of liquid contained in the dish. Diseased leaf tissue was broken into fragments about 5 mm. in length, and the spores were shaken out through the exposed ends of the unopened sori. There was practically no contamination by other fungi. This method resulted in saving of time and made it possible to use material from the same source in a series of tests.

NATURE OF SUBSTRATE

Various organic and inorganic substrata were used. "Nutrient solutions" of inorganic salts and various sugars, carrot tissue infusions, wheat plant tissue infusions, beerwort, soil water extracts, manure extract solution, and other media were used in a number of different concentrations and under different conditions, without materially increasing the amount of germination.

A number of organic acids were used in an attempt to find some specific stimulus for germination, but without success.

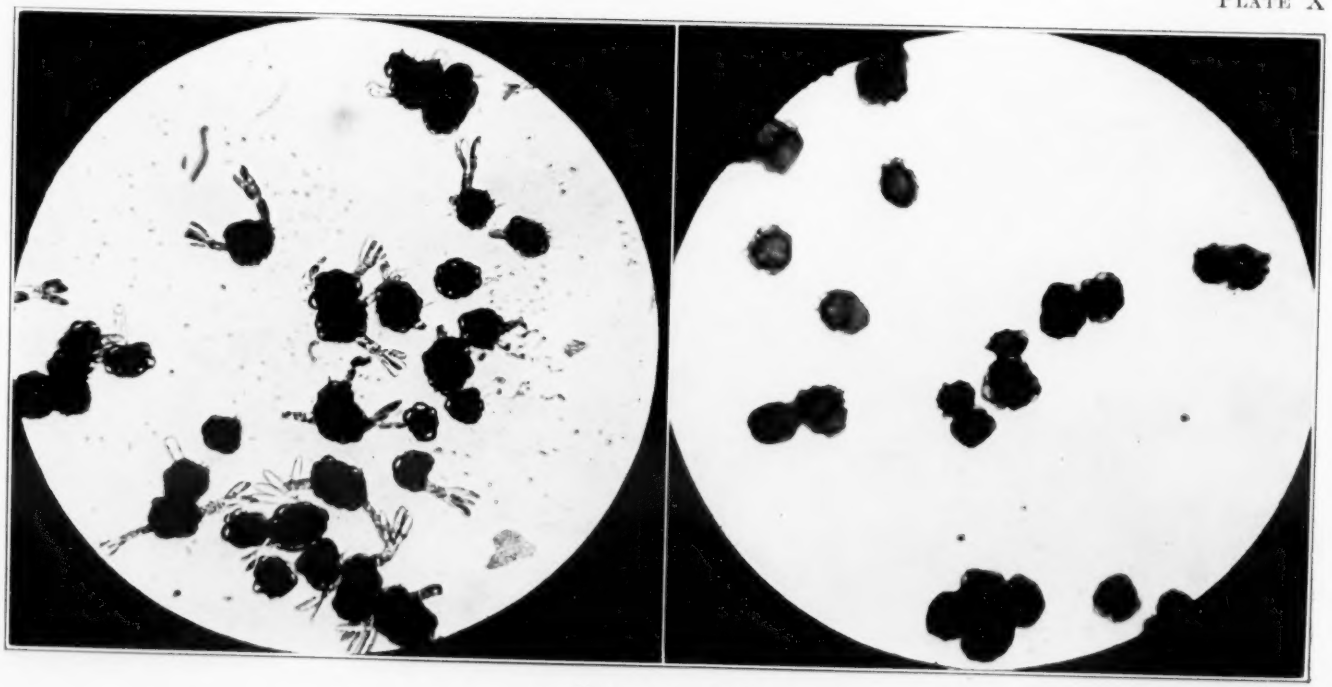
The spores did not germinate readily in any of the sodium phosphate solutions of different hydrogen-ion concentrations. Various soaps of saturated and unsaturated fatty acids were used to change the surface tension of the medium, but none of the changes appeared in themselves to be of great significance.

The spores occasionally germinated in soil water extracts and in plant tissue infusions, but even then only to a slightly greater extent than they did in distilled water. In distilled water at 22° C, a few spores occasionally germinated after 4 days, but sometimes no germination was observed until after 16 days, and frequently all the spores failed to germinate.

Subsequent tests have indicated that the maturation of the spore was not a limiting factor in these cases, for spores which were from 1 to 24 months old germinated under suitable environmental conditions.

No method of spore treatment appeared to stimulate germination consistently, until it was found that spores which had been presoaked in water for several days would germinate profusely after the addition of small quantities of wheat seedlings tissue. (A few razor sections about 20 μ. in thickness.) (Pl. X, fig. 1 and 2.)

It will be noted from table 1 that the stimulatory action of the plant material is much more potent when it is added to spores which have been presoaked for 3 or more days than when it is added simultaneously



UROCYSTIS TRITICI

FIG. 1. Germination of spores of *Urocystis tritici*. Twelve hours after addition of plant tissue to spores presoaked in distilled water. Germination at 25° C. × 190.

FIG 2. Germination of spores of *Urocystis tritici*. Spores presoaked in distilled water as in figure 1. No plant tissue added. × 190

with them. In a number of tests when dry spores were sown on wheat plant infusions, they failed to germinate, whereas presoaked spores germinated readily in the same media. The spores remained in a receptive condition for at least 28 days after sowing in distilled water, although in this case the addition of tissue resulted in only 2 per cent germination.

TABLE I

The effect on germination of adding wheat tissues to distilled water in which spores of Urocystis tritici had been presoaked for different periods of time.

Dish No.	Date on which spores were sown	Date on which tissue was added	Percentage of germination						
			Feb. 27	Feb. 28	Mar. 1	Mar. 2	Mar. 5	Mar. 7	Mar. 8
80	Feb. 24	Control	0	0	0	0	0	trace	
81	"	"	0	0	0	0	0	0	trace
82	"	Feb. 24	0	trace					
83	"	"	0	0	1				
84	"	Feb. 25	trace	2	2				
86	"	Feb. 27	0	30					
87	"	"	0	20					
88	"	Feb. 28	0	0	90				
89	"	"	0	0	85				
85	"	Mar. 1	0	0	0	65			
91	"	Mar. 4	0	0	0	0	70		

All portions of the young wheat seedlings appeared to be equally effective. The addition of uninjured wheat seedlings to dishes of distilled water containing presoaked spores also stimulated germination almost to the same extent as did the addition of small portions of plant tissue.

The action was not specific for wheat plant tissue, since the tissues of rye, barley, oats, flax and various grasses also stimulated germination of presoaked spores, although wheat plant tissue was the most effective.

Tissues derived from seedling wheat plants of the einkorn, emmer, and spelt groups, were all equally effective. No difference was observed between the action of tissue derived from wheat varieties susceptible to flag smut and from those which are resistant.

It was found that autoclaved infusions and also the distillates obtained from water extractions of wheat seedling tissue also effectively stimulated the germination of presoaked spores, provided that satisfactory dilutions were used. Dry spores sown in or on such media frequently failed to germinate.[1]

The general conditions necessary for germination of the spores thus having been established, further studies were then made on the physiological relationships and the morphological characters of the organism.

RELATION TO TEMPERATURE

Fawcett (2) has pointed out that the cardinal temperatures of any organism may vary considerably, according to the conditions under which the determinations are made.

In studies on the temperature relationships of *U. tritici* it was found that the nature of the treatment to which the spores had been subjected had a considerable influence on their subsequent behavior at germination. Strong germination was always obtained 12 to 18 hours after the addition of wheat plant tissue to spores which had been presoaked for approximately 3 days at from 22° to 24° C. (Pl. X, fig. 1). It was found, however, that spores which had been presoaked at lower temperatures and subsequently incubated at 27.5° C. rapidly lost their viability (Tab. 2). The moisture-temperature relationship thus indicated is possibly a factor in causing the reduction of infection, frequently noted when wheat is sown in infested soil some time after the incidence of rain.

Further studies on the temperature relationship were made at a fixed temperature range. The spores were sown on the surface of distilled water in Syracuse dishes and incubated for 3 days at temperatures which ranged from 7° C to 27° C. On the addition of portions of wheat plant tissue, it was found that germination commenced first at 18° C, then it occurred successively at 22° C, 12° C and 7° C, none being noted at 27° C.

[1] Since the preparation of this manuscript, Brown (Studies in the physiology of parasitism IX. The effect on the germination of fungal spores of volatile substances arising from plant tissues. Ann. Bot. XXXVI: 294. 1922) has recorded the stimulatory effect of distillates derived from water infusions of the leaves of Ruta and Pelargonium on the germination of spores of *Botrytis cinerea*.

It was found that there was very little difference in the percentage of germination after continuous incubation within a temperature range of from 19° C to 24° C, but that the growth of germ tubes was a little more rapid at the higher temperatures.

In a number of tests involving the use of spores which had been presoaked at 20° C, it was found that a few spores presoaked for from 6 to 8 days germinated even at 29° C; and, in one case, a few abnormal promycelia were formed at 32° C, but none of the spores germinated at these temperatures after they had been presoaked for only 3 or 4 days.

TABLE 2.

The effect on germination of presoaked spores of Urocystis tritici of incubation for varying periods at 27.5° C. prior to the addition of wheat plant tissue.[1]

Dish No.	No. of hours at 27.5° C.	Percentage of germination at 20° C.
76 C	3	80
76 D	6	72
76 E	17	21
76 F	21	25
26	24	20
27	30	trace
28	36	0
76 G	41	0
29	45	0
66	control[2]	90
30	"	80

With reference to low temperatures, it was found that germination occurred at 5° C, although this test involved the use of ungerminated spores which had been presoaked for 6 days at 20° C (Tab. 3). In one

[1] Spores presoaked for 5 days at 20° C.

[2] Controls at 20° C. throughout. Tissue added to all dishes simultaneously. Dishes removed to 20° C. after incubation at 27.5° C.

test it was found that the sporidia grew slowly even at 0° C. A dish of spores in the earliest stages of germination was placed in a water tight container and then totally immersed in a mixture of ice and water. The sporidia had germinated after immersion for 24 hours.

In a temperature range test with presoaked spores, it was found that the maximum growth of germ tubes took place at a temperature of 24° C, although there was very little difference in the percentage of germina-

TABLE 3

The effect of temperature on the germination of spores of Urocystis tritici[1]

Dish No.	Temp.	Percentage of Germination				
		Apr. 10	Apr. 11	Apr. 12	Apr. 13	
98	5°C	0	0	0	80	Promycelia in early stages of growth
99	5°	0	0	0	75	"
100	13°	0	0	98		"
1	13°	0	0	95		"
2	18°	0	90			Average length of promycelia and sporidia 30μ
3	18°	0	92			"
4	20°	0	90			" 40 μ
5	20°	0	95			"
6	24°	0	85			" 105 μ
7	24°	0	91			"
8	27°	0	82			" 45 μ
9	27°	0	90			"
10	32°	0	trace			Few abnormal germ tubes
11	32°	0	0			

[1]Spores sown on distilled water April 4 at 20° C and transferred to wheat plant decoction and to incubators April 10.

tion throughout the range, 18 to 27° C. Under these conditions the maximum temperature apparently is just below 32° C, and the minimum temperature is between 0 and 5° C. (Tab. 3).

It will be observed that spores germinated vigorously at 27° C; but, from previous tests, it is known that constant incubation at temperatures above 25° C is extremely unfavorable to spore germination. From the practical standpoint, however, it is interesting to note that germination may occur within a comparatively wide range of temperatures, but that the previous history of the spores immediately prior to germination has a considerable influence on their subsequent behavior.

It thus appears that spores which are incubated at fixed temperatures have lower optimum and maximum temperatures for germination than those which are noted for spores which have been presoaked at low temperatures before exposure to a range of temperatures.

Under field conditions the spores are subject to a constantly changing environment, and it is apparent that their behavior must vary considerably from that which might be assumed from laboratory tests, in which the spores are only exposed at fixed temperatures for extended periods.

In the absence of moisture the spores will withstand much higher temperatures than those recorded above for spores in the presence of moisture, although the extreme limits in the former case have not been determined. In the presence of moisture, however, it is noted that those temperatures which permit a period of presoaking at comparatively low temperatures considerably extend the temperature range within which germination may occur.

RELATION TO OXYGEN SUPPLY

In preliminary tests it was found that the spores of *U. tritici* rarely germinated when totally immersed in the medium in which they were sown. This fact is generally attributed to the lack of oxygen, and has been frequently noted by investigators for other types of spores. The results of Melhus and Durrell (4) with urediniospores of *Puccinia coronata* Cda. are quite characteristic of the general experience. Duggar (1) has also referred to the phenomenon in connection with the germination of the spores of certain of the Basidiomycetes.

In tests with spores of *U. tritici* it was found that those which were totally submerged in distilled water would germinate almost as readily, and to practically the same extent, as those which were sown on the surface, on receiving a suitable stimulus after a presoak period.

There was a distinct tendency for the promycelia of the totally submerged spores to become abnormally elongated before producing sporidia. The average length of the promycelium produced by submerged spores was approximately 70 microns, while the average length of the promycelia developed by spores on the surface was approximately 30 microns.

Subsequent growth, however, was more rapid on the surface, where the sporidial germ tubes grew to a length of from 150 to 180 microns before any change was observed in the sporidia produced by the totally submerged spores.

SOME MORPHOLOGIC FEATURES OF THE GERMINATION OF SPORES OF UROCYSTIS TRITICI

McAlpine (3) has described the spore form and also some of the features of spore germination. On germination each spore of the spore ball may produce a promycelium. Frequent splitting of the spore wall was observed (Figs. 1, a, c, d, and f), although this was not noted in all cases. Under normal conditions the promycelium usually attains dimensions of about 20 to 30 by 5 μ, although in one instance the promycelium grew to a length of 70 μ before forming sporidia (Fig. 1, k and Fig. 2, o); then at the tip there are formed a number of small protuberances (Fig. 1, b and pl. X, fig. 1), which elongate to form a whorl of from 1 to 6 sporidia, 2 to 4 being most frequently found. The sporidia are somewhat cylindrical in shape, and, when fully formed, are usually about 30 by 5 μ in size. At first the young sporidia remain aggregated at the tip of the promycelium, but, as growth proceeds, they gradually diverge to form a series of finger-like protuberances. Further growth most frequently is manifested by a prolongation of the sporidium while still attached to the promycelium (Fig. 1, j and l). Sometimes additional "buds" may be formed at the tip of the promycelium at the base of the whorl of sporidia (Fig. 1, e, g, h); and these may be prolonged into germ tubes before any are produced by the first formed sporidia (Fig. 1, l).

These prolongations, which may be considered as germ tubes or infection threads, have been observed to grow to a length of about 900 μ. They are fairly constantly about 2 or 3 microns wide. The protoplasm becomes concentrated at the tips, while, at the same time, the remainder of the mycelium may become multicellular (Figs. 1, j and 2, o). When the septa are formed, however, the cells are devoid of protoplasm.

Fusion of sporidia was noted several times (Fig. 1, d, e, f, and n); and occasionally there also was branching and fusion of the germ tubes (Fig. 1, i).

Under some conditions of germination other irregularities were observed. Occasionally no sporidia were developed, but the promycelium

FIG. 1. Germination of spores of *Urocystis tritici:* a–f, × 450; g–h, × 330; i–n, × 250. (Camera lucida drawings.)

developed into a germ tube (Figs. 1, m and 2, q and u). In one case a secondary promycelium was produced instead of a sporidium. This grew to a length of about 60 μ and then produced a number of protuberances which may be considered as sporidia (Fig. 2, p).

Sometimes, particularly when spores germinated at high temperatures, promycelia were formed but no normal sporidia were produced: the

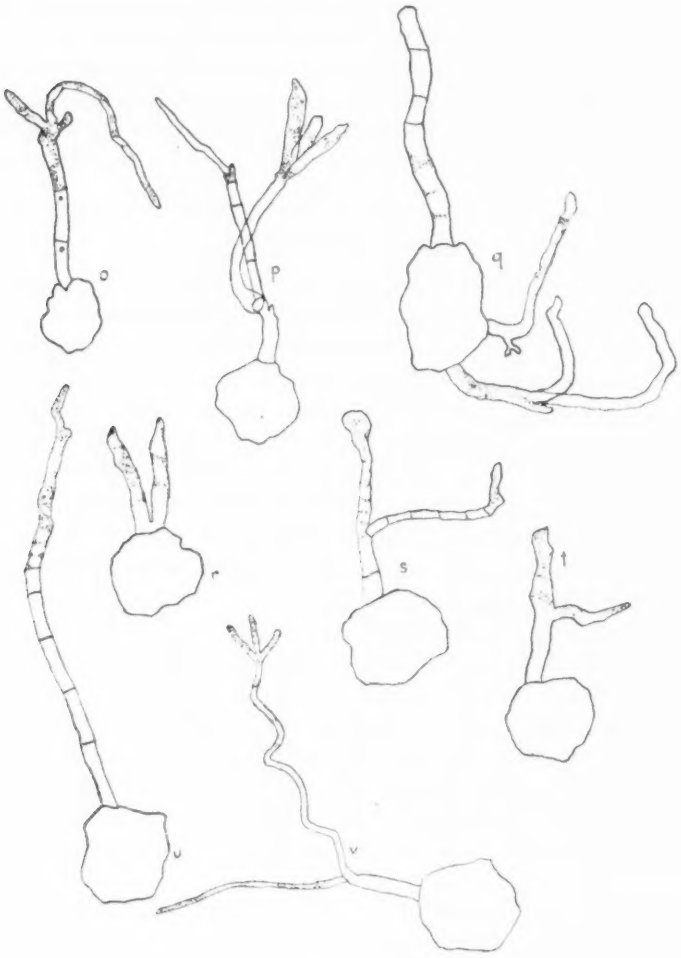


FIG. 2. Germination of spores of *Urocystis tritici*. Less normal type: o, p, r, s, t, v, × 330; q, u × 450. (Camera lucida drawings.)

mycelium merely branched in an irregular manner (Fig. 2, s and t). Several times a portion of the protoplasm was cut off in an offshoot from the promycelium (Fig. 1, g and h).

Thus it is seen that there may be considerable variation on the character of the germination of the spores of *Urocystis tritici*.

DISCUSSION AND CONCLUSIONS

The first prequisite to the development of a disease is the inoculum. It is very evident, however, that susceptible plants may be inoculated with abundant inoculum but yet infection may not occur.

This fact often has been noted with respect to flag smut of wheat. Even though soil may be heavily infested with the spores of the causal organism, a clean crop sometimes may be grown. Under other conditions, however, a relatively small amount of inoculum may be sufficient to cause a serious outbreak of the disease.

The spores of *Urocystis tritici* have been known to germinate capriciously. In fact, it has been difficult to make pathogenicity studies on account of the erratic behavior of the spores.

It has sometimes seemed almost impossible to reconcile the abundant development of smut in the field with the apparent lack of viability of spores. Soil temperature, soil moisture and aeration are known to affect greatly the development of some smut fungi. But the evidence obtained from field observations and from spore germination studies seemed to indicate that these factors alone were relatively unimportant in influencing the infection of wheat by *U. tritici*.

It seemed, therefore, that a sequence of circumstances occurred under natural conditions which were not duplicated in experimental trials. Many unsuccessful attempts were made to hit upon the right combination until it was found that something in plant tissues, and, to some extent in soil infusions, stimulated spore germination in a most remarkable manner. The stimulating substances must be present at the right time and in appropriate concentrations in order to produce the maximum effect. This is indicated particularly by the fact that they are not effective unless the spores have been presoaked. Neither are they effective when the spores have been in water too long nor when they have been kept at an unfavorable temperature. The substances are volatile and appear to be diffusible, at least from the rootlets of young wheat seedlings.

Soil moisture, soil temperature, soil aeration, and the presence of a stimulatory substance at any given time may not be necessarily conducive to infection. It would appear that these factors must operate in the proper order and must be correlated chronologically. Sometimes the factors may operate together in nature in such a manner as to cause the development of flag smut epidemics.

Since the spores of *U. tritici* may retain their vitality in the soil for several years, it is possible that they may be stimulated to germinate by suitable cultural methods; the incorporation of some non-susceptible crop of the right kind in the rotation system, and the practice of "green manuring" might be expected materially to decrease the amount of inoculum in the soil between successive wheat crops.

It is desirable that further studies be made, especially on the factors governing spore germination in the field. Time of planting is frequently of great importance under Australian conditions. Further information on the temperature-moisture relationships under field conditions might reasonably be expected to yield data which would suggest further modification of present cultural practices.

SUMMARY

1. Extensive spore germination studies were made on the spores of *Urocystis tritici* Koern. Practically no spores germinated in ordinary nutrient solutions. At the end of from 4 to 16 days usually only a few spores had germinated in distilled water. Changes in hydrogen-ion concentration and in surface tensions of media appeared in themselves to be of little importance.

2. When small portions of young wheat plants were added to distilled water in which the spores had been presoaked for several days, from 70 to 95 per cent of the spores germinated.

3. Presoaked spores germinated when they were transferred to wheat plant infusions of appropriate dilution or to a distillate obtained from these infusions.

4. The stimulatory substances are volatile, but their exact nature has not been determined.

5. Young tissues of other cereals and a number of other plants also stimulated spore germination.

6. Uninjured wheat seedlings stimulated germination of presoaked spores, as well as did the cut tissues.

7. After spores had been soaked in water for 28 days, they still responded to some extent to stimulating substances.

8. The minimum temperature for spore germination apparently is about 5° C. The germ tubes, however, will grow at 0° C. The maximum temperature appears to be a little less than 32° C. The optimum temperature for spore germination appears to be between 18 and 24° C.

9. The effect of temperature on spore germination varies with the previous treatment of the spores. When a stimulating substance is

added to spores which have been presoaked for 6 days at 20° C the optimum temperature for growth of the germ tubes is about 24° C.

10. Spores which have not been presoaked germinate most quickly at about 18° C. When they are kept at temperatures of over 24° C. for a considerable length of time, they respond less readily to substances which stimulate germination.

11. After total immersion in distilled water for several days at 20° C, the spores germinated readily on the addition of plant tissue. Growth of promycelia was slightly slower than at the surface of the medium.

12. Cultural practices may result in germination of spores in the soil, in a manner similar to that indicated in laboratory experiments.

13. There may be considerable variation in the morphologic features of the promycelia and of the sporidia, according to the conditions under which germination occurs.

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AN IMPORTANT PERIOD IN THE LIFE HISTORY OF TWO BACTERIAL ORGANISMS CAUSING LEAF-SPOTS ON TOBACCO.

W. D. Valleau.

With One Figure in the Text

Angular leaf-spot and wildfire of tobacco are two leaf-spot diseases which have been of considerable economic importance in Kentucky since 1920. In 1920 and 1921, and to a less extent in 1922, these bacterial diseases caused much damage in plant beds and serious losses in the field.

Observations of numerous diseased plant beds and fields indicate that plant bed infection practically always precedes field infection and that disease-free plant beds generally produce disease-free plants in the field. Control of the diseases, therefore, depends on their elimination from the plant bed. The various sources of plant bed infection are not yet definitely known but the work of Fromme[1] leaves little doubt that the organisms may enter the plant beds on the seed. It is also likely that they may be introduced on the feet of laborers who have been working in infected tobacco trash, as Clinton and McCormick[2] have shown that the wildfire organism may live over winter in cured tobacco. We have some evidence that the angular leaf-spot organism, at least, may live in the soil for considerable periods of time.

Because of the numerous possible sources of infection, seed treatment as suggested by Fromme cannot be expected to be efficient except under well controlled conditions. These are hard to obtain when the preparation of the beds is left to untrained help. As a consequence, it is desirable that methods of control be devised which will prevent development of the organisms in the plant bed entirely, no matter how introduced, and thus completely prevent plant infection.

Spraying and dusting with various copper compounds[3] have been suggested as means of control and appear to be effective to a considerable

[1] Fromme, F. D. and S. A. Wingard. Treatment of tobacco seed and suggested program for control of wildfire and angular-spot. (Abstract) Phytopath. **11**: 48–49. 1921.

[2] Clinton, G. P. and F. A. McCormick. Wildfire of tobacco in Connecticut. Connecticut Agric. Exp. Sta. Bull. 239: 365–423. *Pl. 29–32.* 1922. Literature, p. 422–423.

[3] Chapman, G. H. and P. J. Anderson. Tobacco wildfire. Massachusetts Agric. Exp. Sta. Bull. 203: 67–81. *Pl. 1.* 1921.

degree at least. In order to be entirely satisfactory, control must be complete, as the introduction into the field of only a few plants infected with either the wildfire or angular leaf-spot organisms appears to be sufficient, if weather conditions are favorable to spread, to infect large plantings. In other words, the treatment should prevent absolutely the appearance of the diseases on the plants. This necessitates a more accurate knowledge of the life history of these organisms in the plant bed.

The purpose of this note is to record the observations of the writer on the method of primary infection of young tobacco plants in the plant bed and its bearing on the life history and control of these two leaf-spot diseases.

During the past three years the writer has observed numerous cases of wildfire and angular leaf-spot in tobacco plant beds and has had occasion to study the process of infection of young plants. These observations have given little or no information as to the original source of the organisms in the beds, but it may fairly be assumed that they were either present at the time the seed was planted or were introduced at that time, as in Kentucky the beds are hardly touched between the time of sowing and the first appearance of the diseases.

The first infections nearly always occur simultaneously on a considerable number of plants in a small area in a bed or possibly in several similar areas in the bed. They usually appear when the plants are about the size of a quarter. At this time the largest leaves have spread out enough so that their tips just come in contact with the moist soil. It is at this point of contact that infection occurs.[1] These observations suggested that the causal organism was introduced into the soil in spots, possibly on a seed or a small piece of tobacco trash, or in some other way, and that the bacteria rapidly multiplied in the soil and gradually spread out in more or less of a circle during the time when the plants were developing to a size where infection would be possible. As soon as the tips of the leaves come more or less permanently in contact with the infected soil they become diseased, resulting in a rapid soft rot of the tip portion of the leaf. From this time on, secondary infection seems to be purely the result of mechanical conditions—splashing, insects, movements of leaves, etc. The organisms may be readily spread over the remainder of the bed by flowing water.

As the primary plant infections seem always to result from contact of the small leaves with diseased soil, it was thought that control might

[1] Clinton and McCormick (l. c.) have figured and described this stage of the wild fire disease on young plants.

be effected through the addition of toxic materials to the soil early enough to prevent completely multiplication of the organisms in the soil. Consequently an experiment was planned in which sulfur was to be used as the toxic agent. A plant bed was prepared on October 23, 1921, in the greenhouse. Four plots were laid off in this bed. The first received no sulfur, the second sulfur at the rate of 500 pounds per acre, the third at the rate of 1000 pounds and the fourth at the rate of 2000 pounds per acre. Flowers of sulfur was used. It was thoroughly mixed with the upper ¼ to ½ inch of soil *before* the seed was sown. The seed used was a mixed lot of Burley seed obtained by recleaning the waste seed carried over in the process of cleaning seeds for numerous growers. If the seed carries infection, such a lot would be expected to be diseased and so it proved to be. This seed was sown in the four plots. Two other plots were planted in the same bed. One was sown from seed which had been obtained from a commercial seed grower who did not practice bagging the seed heads, thus exposing the pods to infection. The other plot was planted with a root-rot resistant strain of White Burley from a bagged seed head.

Germination proceeded normally in all the plots. After the seedlings were well established but before the production of the first true leaf, some plants in each of the plots damped off. The number of plants which died was in direct proportion to the amount of sulfur in the beds. In those with none, a few plants died. In the bed treated at the rate of 500 pounds per acre, the stand was reduced to 75 plants; where 1000 pounds was used, 25 plants lived, whereas in the plot treated at the rate of 2000 pounds, only 7 plants remained alive.[1] In the untreated control plot about 200 plants were present. The stand in plants from commercial seed was about ⅓ that of the control plot and about identical with that in the 500 pound plot. The various plots were separated from one another by ordinary laths set on edge and buried about half way in the soil. This was not sufficient to prevent some splashing from plot to plot while watering and no particular care was used in this regard.

The first bacterial spots were noticed on December 21, 1921. At that time diseased plants were found in the untreated control plot and in the untreated plot planted with commercial seed. No infected leaves were

[1] The effect on soil micro-organisms was also very striking in the treated plots. In the untreated plots the green and blue-green algae common in greenhouses formed a thin mat over the surface of the soil while they were completely inhibited in the treated plots with the exception of two or three small spots not over two inches in diameter where some growth took place. In figure 1, this effect can be clearly seen in the different color of the soil of the three plots shown.

found in the three treated plots or in the plot planted from bagged seed. The plots were kept under observation until January 12, 1922. Not a single suspicious spot was found in any of the three treated plots, in spite of the fact that there was constantly a chance of the organisms being washed in from the neighboring diseased plots. The toxic action of the sulfur continued to protect these plots from soil infection. Toward

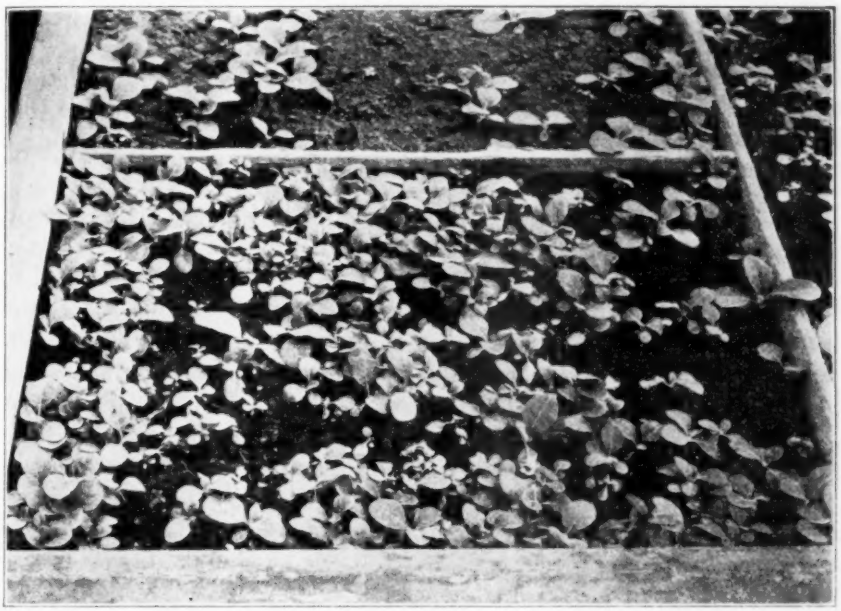

FIG. 1. Photograph taken December 27, 1921, of the untreated control plot showing numerous diseased leaves in contact with the soil. In the rear a portion of the plot treated with sulfur at the rate of 500 lbs. per acre. At the right a portion of the untreated plot planted with commercial seed. A few diseased plants may be seen in it.

the end of the period of observation a few spots had appeared on the plants grown from bagged seeds but bacteria were not demonstrated in any of those examined. Apparently the bagged seed were free from infection when sown and the soil in this plot, therefore, remained undiseased at least until the plants were beyond a stage where leaf-tip infection could readily occur. The diseased leaves in the two infected plots became more numerous, making the plots conspicuous because of the unhealthy appearance of the plants.

The relation between the primary leaf infections and soil infection was very marked in the two diseased plots. The infections could readily

be traced to contact of the first, second, third or fourth leaf tips with the surface of the soil. In the treated plots, although the leaves were as frequently in contact with the soil as in the other plots, no infection occurred and the leaves remained healthy (Fig. 1). In the two diseased plots, the plants at one time were nearly defoliated, but later as new leaves were produced which were more erect, the plants gradually assumed a healthier appearance as is shown in figure 1.

Because of the injury to the stand in the treated plots, the writer does not recommend sulfur as a means of controlling these diseases in the seed bed, but the fact seems to be established that soil infection occurs first and leaf infection follows, and that complete control of the diseases may be obtained by applications of toxic materials early enough to prevent multiplication of the organisms in the soil.

KENTUCKY AGRICULTURAL EXPERIMENT STATION.

PHYTOPATHOLOGICAL NOTES

Sereh disease of sugar cane in Singapore. In the Singapore Botanic Gardens on September 19, 1922, the writer observed sugar cane with shortened internodes and with abnormal development of aerial roots from the nodes. On cutting into the cane, short red streaks were observed at the nodes. The leaves of the affected cane were not streaked or wilting as is the case with cane affected with the Java gum disease. The disease was similar in every way to the sereh disease of Java. Only one cane stool was found to be clearly affected.

Inquiry developed that the canes for this field had been introduced from Java.

To the writer's knowledge this is the first definite observation of the occurrence of sereh outside of Java, although it is known that Java canes have been introduced into Formosa and the Argentine Republic. It is evident that there are still a few cane breeders and sugar producers who are not aware of the risks which they run in importing canes from the oriental countries; in addition to sereh, Fiji disease, downy mildew and cane smut are destructive diseases of sugar cane in the Orient which are not general in the western hemisphere as yet.

Fiji disease especially is a devastating disease under many conditions, equal in effect to such diseases as chestnut blight, pear blight, or pineapple wilt.

The possible introduction of these diseases into the western hemisphere would mean not only financial losses to American sugar interests but ultimately increased cost of sugar to the American consumer.—H. Atherton Lee.

Mottle-necrosis of sweet potatoes. For several years the writers have collected and received specimens of sweet potatoes from various parts of the United States which showed a brown, irregularly formed discoloration of the sweet potato as shown in cross section in figure 1. This mottling appearance characteristic of this disease has been observed at digging time. The patches of dead chocolate-brown tissue are more or less irregularly located throughout the potato. Some of the patches appear to be completely isolated, that is, they have no connection with any of the other dead areas in the potato. In mild cases of this trouble there is no evidence on the surface of any internal disorder. However, in more severe or advanced cases of the disease there are dead areas at the surface. These dead surface spots may occur at the end or at any

point on the surface between the two ends (Fig. 1). A series of cross sections show that nectoric tissue may be found in any part of the potato.

The exact cause of mottle-necrosis of the sweet potato is not known. It was especially bad in the vicinity of Washington, D. C., in the fall of 1922 on the Triumph variety. A less amount was found on the Little Stem and Big Stem Jerseys.

FIG. 1. Mottle-necrosis of sweet potatoes. Surface and cross section views at left and right respectively.

A large number of plantings in agar plates were made from the dead spots. In most cases no organism was obtained from these plantings. A few gave bacteria, a species of Penicillium and one or two species of Fusarium. Plate plantings from specimens collected by one of the authors collected in North Carolina in 1918 gave *Fusarium oxysporum*. Inoculation experiments with this organism gave negative results, however. So far the writers have been unable to demonstrate the presence of any mycelium in the necrotic tissue in isolated spots within the potato. Fungi, however, have been found in the dead tissue in contact with or near to the surface. This disease is being further investigated by the writers.—L. L. HARTER, J. I. LAURITZEN, J. L. WEIMER.

Internal break down of sweet potatoes. Each year the writers have received and collected sweet potatoes which are more or less pithy. A cross section shows that many of the cells of the potato have been pulled apart. The resulting cavities give a more or less cottony appearance to the potato. There is little or no discoloration of this tissue. Cultures from such tissue have always given negative results.

A microscopic examination of such material shows that much of the starch in the vicinity of the ruptured cells has been used up. Internal break down is most prevalent and conspicuous after the potatoes have been several weeks in storage. Some varieties seem to be more suscept-

ible than others. The trouble seems to be associated with conditions of storage. It is more prevalent when the house has been kept fairly dry. —L. L. HARTER, J. I. LAURITZEN, J. L. WEIMER.

Lightning injury to tomatoes. An interesting case of lightning injury was found in an experimental tomato field of the Purdue University Agricultural Experiment Station near LaFayette, Ind., on July 7, the second day after a severe electrical storm. Evidence that the lightning had struck in this vicinity was furnished by the occupants of a farmhouse at one side of this field. The injury to the tomatoes occurred in a more or less circular area about forty feet in diameter. The plants at the center were killed and the severity of the injury became less toward

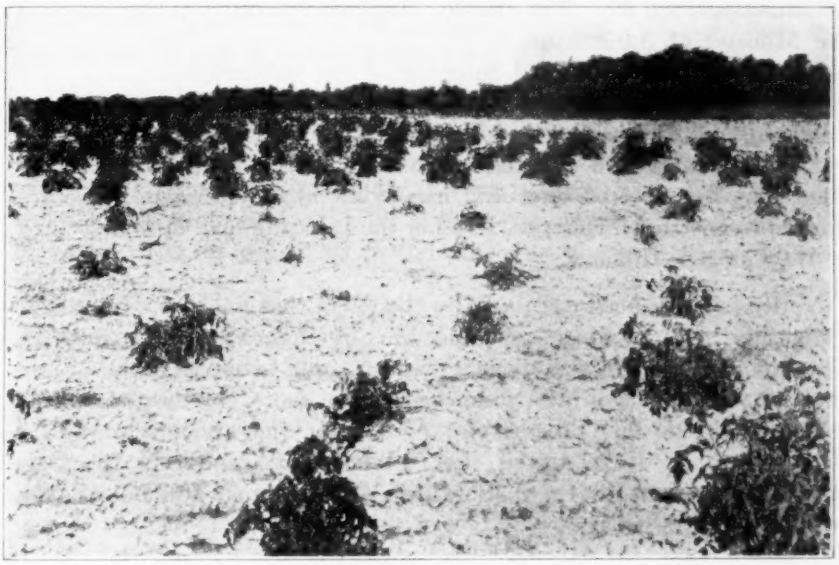

Fig. 1. Lightning injury to tomatoes.

the outer limits of the area. The interesting feature was that the injured plants showed, among other symptoms, typical "hollow-stem," a condition characterized by the collapse of th epith in the stem.—H. D. BROWN AND MAX W. GARDNER.

An international conference of phytopathologists and economic entomologists will be held in Holland from June 25 to 30, 1923. Those wishing to attend are urged to arrange their trip so as to be at Wageningen on June 24. The committee, with Dr. H. M. Quanjer, president, de-

sires that those wishing to read papers send a short abstract to the secretary, Mr. T. A. C. Schoevers, Villapark 8, Wageningen.

The committee has arranged the following provisional program:

Monday, June 25. Presidential address of welcome and lecture on plant diseases. Demonstration of experiments, especially on potato diseases.

Tuesday, June 26. Address by Mr. van Poeteren on organization and methods of the Dutch Phytopathological Service.

Wednesday, June 27. Field trips.

Friday, June 29. Meeting at Baarn. Address by Prof. Westerdijk on the cultivation of parasitic fungi and demonstration of research. Discussion and inspection of the international collection of fungi cultures.

Saturday, June 30. The Hague. Informal meeting at the office of the Minister of Agriculture.

English will be the official language of the conference.

The committee in charge consists of Professor Westerdijk, Mr. v. Poeteren, and Dr. v. Slogteren in addition to Dr. Quanjer.

A cordial invitation is extended to Americans. Detailed announcement may be expected later and meanwhile any inquiries may be addressed to Dr. H. M. Quanjer, Head of the Institute for Phytopathology at Wageningen, Holland.

Notice has just been circulated to the effect that Saccardo's "Sylloge Fungorum" is to be continued under the direction of Dr. Montemartini. The first volume of the Sylloge Fungorum was issued in 1882. Since then 22 volumes have been published. Two more volumes have been completed by I. B. Traverso and A. Trotter and are now ready for publication. It is requested that publications, especially those on mycology, be sent to the Laboratorio Crittogamico Italiano, Pavia, Italy.

Officers for the western division of the American Phytopathological society, which met in Salt Lake City, were elected for the coming biennium as follows:

President, S. M. Zeller, O. A. C., Corvallis, Oregon; Vice President, J. P. Bennett, University of California, Berkeley, Calif.; Secretary-Treasurer, C. W. Hungeford, University of Idaho, Moscow, Idaho; Councellor, representing the Pacific Division, F. D. Heald, Washington State College, Pullman, Washington.

Personals.—Dr. H. M. Jennison, for several years assistant professor of botany at the Montana Agricultural College, Bozeman, has accepted

a position as associate professor of botany in the University of Tennessee, Knoxville. He transferred to his new position September 15.

Prof. G. R. Bisby, Professor of Plant Pathology at Manitoba Agricultural College, has returned to Winnipeg after a year spent at the Imperial Bureau of Mycology, Kew, England, where he has been associated with Dr. E. J. Butler.

Dr. G. R. Lyman, plant pathologist in charge of the Plant Disease Survey of the U. S. Department of Agriculture, has been appointed Dean of the College of Agriculture of West Virginia University, Morgantown, West Virginia, where he will have supervision of the three divisions of agricultural work of that institution, which include the resident instruction in the College of Agriculture, and the work of the Agricultural Experiment Station, and of the Extension Service. Dr. Lyman will enter upon his new duties on January 16, 1923.

Mr. Philip Brierley of St. Paul, Minn., a graduate of the New Hampshire State College, and the University of Minnesota, has been appointed Junior Plant Pathologist, effective July first. He will have headquarters in Washington and will assist Mr. Freeman Weiss in the investigations of wart disease of potatoes. Mr. Brierly has been employed previously by this Bureau for temporary periods in connection with work on blister rust control.

THE FOURTH ANNUAL SUMMER FIELD MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The fourth of the series of annual field conferences of the American Phytopathological Society was held in 1922, in the important trucking sections of southern Delaware, New Jersey, and near Philadelphia, from August 29 to September 1. The convention was given over primarily to the study of diseases of vegetables, the other three annual summer meetings of the Society, held in 1919, 1920, and 1921, being planned with especial reference to potatoes, fruits and cereals, respectively.

The members of the conference gathered at Seaford, Delaware, on the afternoon and evening of August 28, and the next day Dr. J. F. Adams, of the Delaware Experiment Station, conducted the party on a field trip in the vicinity of Seaford. The new and up-to-date sweet potato storage house of the Johnson Potato Storage Company was inspected. This is probably the largest sweet potato house in the country, it having a capacity of 125,000 five-eighths bushel baskets. Other stops were made at farms where crops of cantaloupes, tomatoes, asparagus, cowpeas, soy beans, and especially sweet potatoes were seen. Of the sweet potato diseases, white rust (*Albugo ipomoeae-panduranae*) was very conspicuous and causing more damage than is usually the case with this disease. Fusarium wilt (*Fusarium batatatis* and *F. hyperoxysporum*), black rot (*Sphaeronema fimbriatum*), and leaf spot (*Septoria bataticola*) were seen, and extreme fasciation of the stems of sweet potatoes was very common in many fields. In the various sweet potato fields visited, the members had the opportunity to observe the influence of seed treatment and crop rotation on disease prevalence, as well as to note results being obtained from the use of different varieties and fertilizers. An experimental field of cantaloupes, badly damaged by hail and downy mildew (*Pseudoperonospora cubensis*) was observed. Downy mildew was very severe in Delaware this season, at least 50 per cent of the crop being lost, according to Dr. Adams. In tomatoes Septoria leaf spot (*Septoria lycopersici*), Fusarium wilt (*Fusarium lycopersici*), and mosaic (undetermined) were common. The tomatoes of the vicinity were in rather poor condition owing to wet weather and disease. Diseases of other crops seen were asparagus rust (*Puccinia asparagi*) and the leaf spots of cowpeas caused by *Cercospora cruenta* and *Amerosporium oeconomicum*. A visit was made to a large canning factory at Seaford, where an average of 75,000 cases of peas and 100,000 cases of catsup, chili sauce, and tomatoes are packed annually. Following this, the party left for Philadelphia.

The next day, August 30, was "New Jersey Day" with the program in charge of Dr. Mel. T. Cook. Automobiles conveyed the party from Camden to Glassboro, stopping at numerous points on the way. On the farms of the Campbell Soup Company near Riverton, experiments for the control of Septoria leaf spot and other diseases of tomatoes by the use of Bordeaux mixture and anhydrous copper dust were inspected. At Mt. Laurel, sweet potato varieties grown on soil heavily infested with Fusarium were being compared for resistance to Fusarium wilt, and the relation of the prevalence of this disease on soil type and crop rotation was being demonstrated. One of the most interesting stops of the morning was at Marlton, where in a large plantation of okra the wilt caused by *Verticillium albo-atrum*, and certain leaf spot diseases of this crop were observed. The Verticillium was very abundant, affecting many plants and cutting down the yield to a great extent. Inadequate crop rotation in recent years has evidently favored the increase of the disease. At Glassboro, the Repp Brothers

Company cold storage plant and orchards were inspected. In part of the latter the Insecticide and Fungicide Board of the U. S. Department of Agriculture was conducting some of its official tests with various commercial fungicides, and the effects of some of these classes of spray materials on trees were noted.

On the evening of August 30, specialists of the U. S. Department of Agriculture spoke on the subject of marketing and market pathology. Mr. F. G. Robb, of the Bureau of Markets, discussed the various ways by which fruits and vegetables pass from the producer to the consumer, and explained the operation of the fruit and vegetable inspection service that has been conducted by the Bureau of Markets for the past five years. Mr. F. C. Meier of the Bureau of Plant Industry in his talk which was illustrated with a number of beautifully prepared lantern slides, mentioned the losses from decay that are incurred between the field and the market. He told of the methods of training the market inspectors to distinguish between the various decays and discussed some of the important problems that arise in connection with the work. Dr. L. L. Harter of the Bureau of Plant Industry in his paper on "Research problems in plant pathology and their relation to the transportation and marketing of perishable vegetables," brought to the attention of the conference some of the research that already has been done on the relation of storage conditions to the development of decay-producing organisms. He showed the need for further investigation of this kind, and emphasized the great desirability of further cooperation between the plant pathologist and the market investigator.

August 31, "Market Pathology Day," which was spent entirely in Philadelphia, was full of interest. Under the leadership of F. C. Meier the party visited the Pennsylvania Cold Storage and Market House at 30th and Chestnut Streets; the Dock Street Wholesale Market; the auction piers of the Pennsylvania and Baltimore and Ohio railroads, located at piers 11–15 on the water front; the auction market operated in connection with the piers; the Philadelphia offices of the Bureau of Markets of the U. S. Department of Agriculture; and the Reading Terminal Market and cold storage rooms. This series of inspections gave the members of the conference an excellent idea of the complicated process of marketing fruits and vegetables from the time they come into the city until they reach the hands of the consumer. They were also able to familiarize themselves with the important work that is being done by the Bureau of Markets of the U. S. Department of Agriculture.

"Bustleton Day," September 1, was in charge of Dr. W. S. Beach of the Pennsylvania Agricultural Experiment Station. In the automobile trip through the extensive trucking sections near Bustleton, a great variety of vegetable crops were seen,—celery, rhubarb, spinach, lettuce, Lima bean, cantaloupes, horse-radish, parsley, tomatoes, cabbage, and sweet corn, as well as numerous other truck crops being grown there in large quantities. On account of this diversity of crops a large number of diseases were to be seen. Among the commonest were late blight (*Septoria petroselini*) and early blight (*Cercospora apii*) of celery; crown rot (*Phytophthora cactorum*), leaf spot (Phyllosticta) and anthracnose (Colletotrichum) of rhubarb; pod blight (*Diaporthe phaseolorum*), downy mildew (*Phytophthora phaseoli*) and other pod and leaf spots of the Lima bean; black rot (*Bacterium campestre*) and soft rot (*Bacillus carotovorus*) of cabbage and lettuce drop (*Sclerotinia spp.*). A visit was made to the Research Laboratory of the Pennsylvania Agricultural Experiment Station at Bustleton where Dr. Beach explained the work that is being conducted. Lettuce plants artificially inoculated with *Sclerotinia minor*, and rhubarb infected with different species of Phytophthora, were of especial interest at the laboratory. Experiments for the control of celery leaf diseases

(*Septoria petroselini* and *Cercospora apii*), downy mildew of Lima bean (*Phytophthora phaseoli*), lettuce drop (*Sclerotinia minor* and *S. libertiana*), and rhubarb crown rot (*Phytophthora cactorum*) were inspected.

As was the case with the other annual field conferences, the society was fortunate in having present a number of plant pathologists and botanists from other countries. Dr. I. B. Pole-Evans, Chief of the Division of Botany, Department of Agriculture, Pretoria, Union of South Africa, and Senor Alfonso Madariaga, of the Department of Agriculture of Mexico, were officially designated by their governments to come to this country for the purpose of attending the conference. Other visitors of note were Dr. Seiya Ito, Hokkaido Imperial University, Sapporo, Japan, Dr. Takewo Hemmi, Kyoto Imperial University, Kyoto, Japan, Dr. Rudolph Kuraz of the Czechoslovakian Legation, Washington, D. C., Mr. Carlos A. Vallejo of the Argentine Embassy, Washington, D. C., Mr. R. J. Noble of Australia, Mr. N. G. Teodoro, Department of Agriculture and Natural Resources, Manilla, Philippine Islands, Mr. G. O. Ocfemia, College of Agriculture, Los Banos, Philippine Islands, Dr. C. Boyle of the Department of Agriculture and Technical Instruction for Ireland, and Mr. D. Borodin of the Russian Agricultural Bureau, New York City. The meeting was full of interest and those who attended, between sixty and seventy-five in number at various times, found it an extremely profitable occasion. The pathologists of Delaware, New Jersey, and Pennsylvania, who had the program in charge, the local growers, county agents, and others, who assisted by furnishing automobile transportation and in perfecting arrangements, the officials of the Pennsylvania Railroad, the Reading Terminal Market, and the U. S. Bureau of Agricultural Economics, who rendered valuable service on Market Pathology Day, all deserve much credit, and have the thanks of the entire Society for making the meeting such a complete success.—R. J. HASKELL, Secretary, Advisory Board, American Plant Pathologists.

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